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PROCEEDINGS OF SEMINAR ON METHODOLOGY FOR MONITORING THE MARINE ENVIRONMENT, HELD IN SEATTLE, WASHINGTON, OCTOBER 1973

S. S. Verner

Environmental Protection Agency
Washington, D. C.

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This document contains the proceedings of the "Seminar on Methodology for Monitoring the Marine Environment" held in October 1973. The Seminar was organized to strengthen the Agency's coastal monitoring program and focused on physical-chemical biological, and microbiological techniques for assessing the quality of marine and estuarine waters.

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EPA-600/4-74-004
October 1974

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OF
SEMINAR ON METHODOLOGY
FOR
MONITORING THE MARINE ENVIRONMENT

SEATTLE WASHINGTON
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FORWORD

The Seminar on Methodology for Monitoring the Marine Environment was organized to assist the Environmental Protection Agency in establishing a basis for common methodological techniques in marine and estuarine water quality management. The meetings focused on procedures for sampling and analyzing coastal waters involving the physical-chemical, biological, and microbiological disciplines. The Seinar had a three fold purpose: to serve as a forum for presenting state-of-the-art technology in environmental quality monitoring of saline and brackish waters; to present recent developments in sampling and analysis of these waters; and to clarify and identify problem areas and desirable research goals.

The Seminar was open to anyone with an interest in techniques for monitoring the environmental quality of coastal regions. The meeting was necessarily of limited scope and additional seminars may be useful in the future. The Office of Monitoring Systems welcomes comments on these proceedings as well as suggestions for future activities.

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PREFACE

This document contains the proceedings of the "Seminar on Methodology for Monitoring the Maring Environment" held in October 1973. The Seminar and these proceedings are representative of a continuing effort by the Office of Monitoring Systems to highlight issues and alleviate, through inter and extra Agency-wide cooperative efforts, problems of monitoring environmental quality requiring technical or administrative support.

This Seminar was organized to strengthen the Agency's coastal monitoring program and was divided into three principle segments, viz., basic framework and field applications, monitoring for pollutants which concerned physical-chemical and biological techniques for investigating eight classes of pollutants; and microbiological aspects for assessing the quality of marine and estuarine waters. The theme of the meeting was set by Dr. Stanley M. Greenfield, Assistant Administrator for Research and Development. A final paper by Dr. Donald Squires of the New York State Sea Grant Program addressed the problem of relating monitoring systems to the users of environmental data.

INTRODUCTION AND THEME

Stanley M. Greenfield *

I would like to add my words of welcome to those of the Chairman in wishing you a fruitful and productive meeting.

We have organized this seminar to bring together those concerned with monitoring the quality of marine and estuarine waters; to engage in a free exchange of ideas; to learn firsthand about progress in the development of methodology; and hopefully to come away with a better understanding of current technology for coastal and near coastal regions. Perhaps of equal importance are the bonds we can develop between the academic community and EPA.

Monitoring of the marine environment is an integral part of environmental management and the control of pollution, since the Administrator of the Environmental Protection Agency is thus provided with information for decision-making relating to the state of the environment, trends, compliance with standards, etc. Environmental monitoring data is also essential for assessing and evaluating control strategies and abatement measures as well as status and trends in the quality of the marine environment. The assessment of environmental quality is vitally important in determining national environmental policy and in keeping the public informed regarding the condition of the environment.

I should take a moment to explain what I mean by environmental monitoring. What is meant is the systematic and continuing observation of environmental quality parameters including physical-chemical, biological and microbiological techniques for the purpose of providing a sound data base for EPA's criteria development, regulatory and operating programs. The scope of a monitoring program consists of: (a) the development of sensor devices, instrument systems and analytical procedures; (b) sample collection and analysis; and (c) data and information storage and retrieval for the detection and measurement of pollutants and the assessment of environmental quality.

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*Formerly Assistant Administrator for Research and Development, U. S. Environmental Protection Agency.

I need not remind this audience that EPA is basically a regulatory Agency whose primary function is enforcement of environmental quality standards in both air and water established by statute. The monitoring requirements placed on EPA and the States necessitate a sophisticated sensor network extending through all the coastal regions. However, such a network must be well planned and coordinated. The Office of Monitoring Systems under Will Foster's direction is responsible for developing an overall monitoring strategy for the Agency. Some of the important considerations in the development of this strategy are:

- technical, administrative and logistical support for development of monitoring methodologies;
- development of environmental baselines in order to identify environmental quality trends;
- timely incorporation of newly developed monitoring techniques into Agency monitoring networks;
- identification of short-term and long-term monitoring requirements;
- standards for methodology in monitoring applications;
- utilization of state-of-the-art sensors, techniques and procedures;
- a data link between sensors and data storage systems.

These functions spell out the Office of Research and Development's interest and involvement in monitoring environmental quality and form the basis for relationships with other agencies and institutions.

However, all the activities I have enumerated rest on the premise of a vigorous and competent technological program. In fact, I would venture to say that only through a reliable, efficient and timely monitoring program based on the most advanced concepts in technology will EPA be able to meet the challenge of restoring and maintaining a viable marine environment for present and future generations.

Consequently, this meeting was called to further the development of marine monitoring techniques and methodology and the subsequent standardization of methodology which is not yet available and requires a concerted effort by all concerned. We all recognize the pitfalls and difficulties of the conventional, i.e., manual techniques for analyzing and evaluating environmental quality in sea and brackish waters. Unfortunately, traditional time-honored analytical methodologies all too often have not been suited to marine waters and organisms. As an example, not too long ago the National Science Foundation conducted an experiment in which lead was dissolved in sea water to a concentration of 15 ppb; 10 aliquots were distributed to various university and marine institute laboratories for analysis; the results that came back reported concentrations of 50-1500 ppb; subsequent repeats of this experiment have brought results which vary by only an order of magnitude!

When we consider the importance of coastal and near coastal regions to our health and well-being, e.g., as a source of recreation and food, it is apparent that we must bring the level of technology of marine monitoring methodology at least on a par with other monitoring methodologies.

While seeking to improve existing technology, it is also important that we look to the future in our continuing efforts to advance the state-of-the-art. Historically, the routine monitoring of environmental pollutants in marine waters has involved analysis of selected samples collected "in the field". Such sampling techniques are not only time consuming, but more important, they have limited utility considering the vast geographic areas and pollution sources that must be sampled for truly effective environmental monitoring. Clearly, for the future, the routine monitoring of marine and estuarine waters will most effectively be conducted through automated devices operating in the in situ or remote sensing mode and functioning in real-time.

Needless to say, an operational network of such sensing instruments, to be widely deployed and effective, must contain elements which are relatively inexpensive, reliable, sensitive, accurate, pollutant-specific and easily maintained and calibrated. Ultimately, the acceptance of advanced monitoring systems rests on the intrinsic performance of the sensors and instruments and the degree to which they satisfy the aforementioned requirements.

Although many advancements in sensor technology may be expected in the future, improved methodology will always remain a goal and sensor development will continue to be of great concern to us. This is particularly so in areas of electro-optical instrumentation involving correlation or matched filter techniques and derivative spectrometry, and the class of electro-chemical probes using ion selective electrodes where important developments are occurring in sensor technology.

In looking to the future, it is essential that the evolution of sensors be closely followed and supported by EPA because ever newer generations of sensing instruments will radically affect techniques for monitoring the quality of the marine environment.

Assembled at this meeting are some of the leading exponents and proponents of marine environmental science to discuss problems which are fundamental to an effective monitoring program. I am confident that the results obtained here will constitute an important contribution to EPA's future monitoring strategy in the marine environment.

* * * * *

INTRODUCTORY REMARKS TO SESSION A

BASIC FRAMEWORK AND FIELD APPLICATIONS

MODERATOR: DONALD J. BAUMGARTNER*

This session is entitled Basic Framework and Field Applications. This is not an entirely satisfactory descriptive title, but was the closest I could think of which attempted to describe some of the monitoring problems we would consider this morning. In initial planning of this symposium, several titles such as, "Physical", "Hydrodynamic", "Hydrologic" were considered as descriptive of the topics to be considered. of currents, salinity, and temperature for several reasons. First, these systems are rather well developed and are continually being improved by the Navy, the National Oceanographic and Atmospheric Agency, by Oceanographic Institutions, and by the manufacturers of oceanographic equipment. These systems which are routinely employed in oceanographic institutions are well described by the technical literature of the manufacturers and by reports in ocenographic journals. The second reason is that the state-of-the-art of measuring pollutants in coastal waters is not only much more in need of development but is certainly more the province of the Environmental Protection Agency and should receive considerably more attention in symposiums such as this. Two of the papers in this session will deal with pollutant measurements having some physical properties, particulates associated with turbidity, and surface films.

Taking the work physical in another sense, that of a physical science, a paper on the application of mathematical modeling as a fundamental consideration in the design and operation of a monitoring system will be presented.

MATHEMATICAL MODELING AS A FRAMEWORK FOR COASTAL MONITORING

R. J. Callaway *

INTRODUCTION

Several definitions are required as a prelude to this paper. The first few are taken from Webster's Seventh New Collegiate Dictionary.

mathematics	--the science of numbers and their operations, interrelations, combinations, generalizations...
model	--a system of postulates, data, and inferences presented as a mathematical description of an entity or state of affairs.
monitor	--to watch, observe or check, esp. for a special purpose. --to keep track of, regulate, or control.
surveillance	--close watch kept over a person or group (as by a detective).

From these we can suggest that environmental mathematical modeling, as a monitoring and/or surveillance tool, would be an attempt to describe, predict, and generalize on pollution caused transformations and the regulation or control of such events as guided by or inferred from model output.

MODELS, MODELING AND MODELERS

Several classes of models exist, among them: conceptual models which may be the framework of an eventual working model; analytical models which usually result from analytical solutions of simplified (tractable) systems; numerical models of complex (analytically intractable) systems; hydraulic models which are scaled down, usually space distorted, images of real entities or environments;

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* Environmental Protection Agency,
Pacific Northwest Environmental Research Laboratory,
Corvallis, Oregon

analog models which use electronic components as analogues of physical systems and mathematical operations. The main emphasis here is on analytical and numerical models.

It has only recently been recognized that environmental problems occur at the systems level of complexity (12). It is usually the biologist who has the last say as to how detrimental a particular situation is or may become. Since the biologist is rarely a systems engineer, he will do well to consult with the physicist (or engineer) on certain problems thus avoiding Cohen's (4) rule: "Physics-envy is the curse of biology." Likewise, the physicist working on theoretical biological models should harken to Boorman's (2) advice: "Physicists who wish to do useful biology should not try it on their own."

MONITORING SCHEMES RELATED TO THE TYPE OF WASTE INPUT

Figure 1 is a schematic of mathematical formulations of various waste inputs and the response of a system to the input. Here, W is waste load concentration, t is time,   is frequency and s is system response. Following Thomann (11), assume a box or reservoir model in which all constituents of interest are completely mixed. The inputs shown in Figure 1 are discussed from the point of view of the mass balance equation with constant system parameters:

1) $$V \frac{dC}{dt} + QC + KVC = W(t)$$

where

V = volume of system (box), L^3
C = concentration of waste, ML^{-3}
t = time, T
Q = advection through box, L^3T^{-1}
K = reaction (decay) rate, T^{-1}
W = waste load input, MT^{-1}

Instantaneous Input

The solution of equation 1 with W(t) multiplied by an impulse function is

2) $$C = \frac{m}{V} \exp\left[-\left(\frac{Q+KV}{V}\right)t\right]$$

where m = mass discharged instantaneously.

The assumption of a well-mixed box no longer applies as the instantaneous input assumes a point source initial distribution. Since there is no dispersive mechanism (in the equation) the initial distribution decreases with time in the direction of flow but maintains its original (infinitesimally small) lateral configuration.

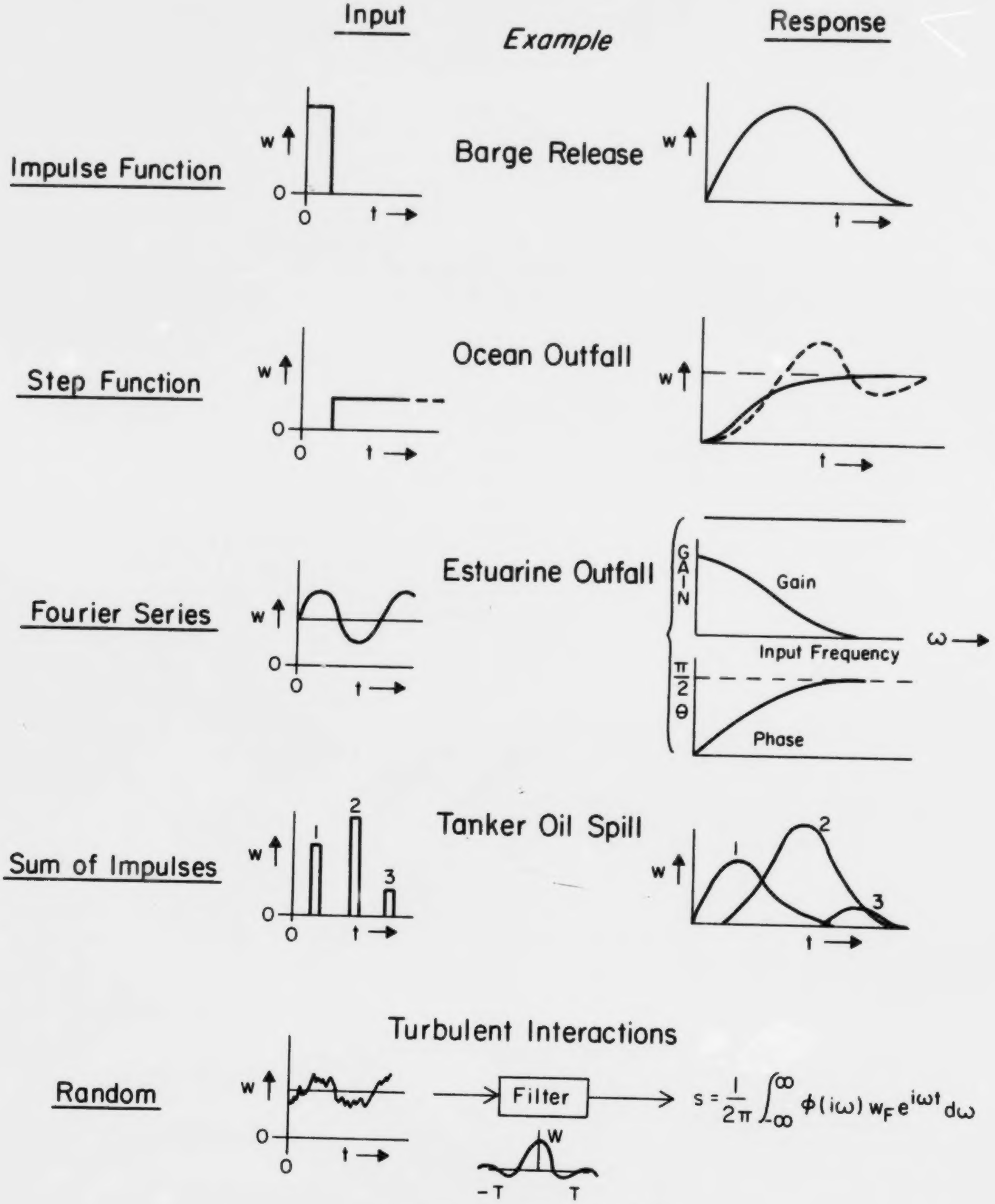

Input
Example
Response
Impulse Function
w
0
0
t
Barge Release
w
t
Step Function
w
0
0
t
Ocean Outfall
w
t
Fourier Series
w
0
0
t
Estuarine Outfall
GAIN
Gain
Input Frequency
ω
π/2
θ
Phase
Sum of Impulses
w
1
2
3
0
0
t
Tanker Oil Spill
w
1
2
3
t
Random
w
0
0
t
Turbulent Interactions
Filter
W
-T
T
s = 1/2π ∫_{-∞}^{∞} φ(iω) w_F e^{iωt} dω

FIG. 1. Schematic input-output relationships

In order to monitor impulse response situation which might be approximated by a barge release of wastes in the open sea, as hoc procedures will most likely have to be employed. These will relate to the necessity of following the release throughout the water column and into the sediment for later monitoring of the effect on benthic communities, etc. Of course, in real situations the identity of the point source cannot be maintained for long; to describe and predict the spread adequately, equations have been developed which include more complex advective and diffusive terms but which still utilize the initial impulse function.

Constant Input

Equation 1 can be easily solved for the step function input as:

$$3) \qquad C = \frac{W}{Q+KV}\left(1 - \exp\left[-\left(\frac{Q}{V} + K\right)t\right]\right)$$

Equation 3 makes some aspects of the monitoring problem become clear: as t becomes large the concentration in the system approaches steady-state i.e., as:

$$t \rightarrow \infty, \; C \quad \frac{W}{Q+KV} \text{ and } \frac{dc}{dt} \rightarrow 0.$$

Concentration at steady-state is directly proportional to the waste input and inversely proportional to the system parameters. Near the source (V-0), or for a conservative substance ($K = 0$), the steady-state concentration is W/Q.

If a given system can be considered as a single well-mixed box with uni-directional flow then a single fixed instrument placed anywhere in V could adequately monitor events with respect to its equilibrium time. In reality, of course, the actual placement of the instrument will be a function of several considerations such as distance from the source, ease of servicing, etc.

Periodic Input

For periodic inputs, the simplest is sinusoidal:
$W(t) = W + W_o\text{Sin}(\omega t + \emptyset)$ where:
- ω = fundamental frequency, T^{-1}
- Φ = phase lag, radians
- W = mean input, MT^{-1}
- Wo = amplitude of sinusoidal input, MT^{-1}

Equation 1) becomes

$$V\frac{dc}{dt} + k = W + W_o\text{SIN}(\omega + \emptyset), \; k = Q + KV$$

At steady-state

$$C = \frac{W + Wo\ SIN\ (\omega t+\phi)}{k}$$

and is seen to be frequency dependent and oscillating about W. The non-steady periodic output response can be shown to be (Thomann, op. cit.):

$$Cp = WoAm(\omega)\ Sin[\omega t+\phi-\theta(\omega)]$$

Where

$$Am(\omega) = \frac{1/V}{[k/V^2 + \omega]^{\frac{1}{2}}}$$

$$\theta(\omega) = Arctan(\frac{\omega}{k/V})$$

The system parameters K, Q, V and the input frequency, w, determine the amplitude and phase characteristics of this particular system. In general, for any given input which can be approximated reasonably well by a Fourier series the total response of the well-mixed system will consist of a mean response plus periodic responses for each series term. To evaluate the results, Bode or Nyquist plots are usually required. For the results above, however, it can be seen that an increase in input frequency results in an asymptotic decrease in A to zero; $\theta(\omega)$ approaches $\pi/2$ asymptotically. The equilibirium response of the system is

$$C = \bar{C} + C_p$$

where

$$\bar{C} = W/k$$

Knowledge of the input frequency would be required for determining instrument response times if the input frequency is high, i. e., the instrument response time and sampling frequency at a given station would need to be such that effluent peaks are not filtered out. In order to relate the instrument reading to the source the phase lag due to frequency shift needs to be related to the distance from the source. For simple flow-through, constant Q systems this is an easy task; however, for complex tidally varying systems it is a difficult problem requiring time-series analyses. For routine purposes this analysis will not usually be feasible or necessary. Where it is necessary, design criteria for the sampling period need to be met. These criteria relate to the period of the expected dominant frequencies, usually tidal, in the water body.

Arbitrary Input

A stationary tanker leaking oil at different rates with time can be considered as an arbitrary input case. If the rate of discharge varies about some mean in an aperiodic fashion, special mathematical techniques need to be employed because techniques associated with periodic phenomena are inapplicable.

An arbitrary input, u(t) can be thought of an consisting of n unit impulses each with its own amplitude, a , occuring at time t :

$$U(t) = \sum_{n} a_n \delta(t-t_n)$$

The response to each impulse is R(t-t), a weighting function which is determined by the system parameters (Q, V, K). This function weights all previous inputs up to the time of observation with the most recent inputs having the greatest weight. Formally,

$$X\ (t) = \int_{-\infty}^{t} u(t_1) R(t-t_1)\ dt_1$$

As time t may be expressed as distance from the source then t = y/v, where y is distance from the source. Here, v = Q/A = flow through a uniform cross-sectional area and the convolution integral can be thought of in terms of a steady-state velocity with the weighting function having less weight with distance from the source.

Stochastic Input

It may be required to monitor an aperiodic or stochastic input or a continuous output record such as, dissolved oxygen in an estuary. Certain periodicities in the D. O. record will be obvious and related to tidal periods and diurnal and semi-diurnal fluctuations, however, the effluent discharged from an outfall may have a stochastic rate of discharge. Thus, it is implied that two mechanisms may account for a stochastic output: stochastic waste input and/or physical and biological processes in the environment. The analysis and synthesis of these events, i.e., the analysis of the contribution of the various processes of waste in order to synthesize the output record, can be accomplished to some degree, although a complete interpretation will rarely be attained.

It is unlikely that anything less than a continous or closely spaced record of the parameter under consideration will lend itself to the computations required for analysis of a stochastic record. If it is known that it will be necessary to monitor and analyze a stochastic event then special instrumentation and field procedures will have to be developed with a clear understanding of what is to be sampled and when. An excellent source of information on the time-series analysis required is given in Bendat and Piersol (1). Not of small importance is an understanding of what the payoff will be and a determination of whether the payoff justifies the field investigation and subsequent analysis.

MONITORING IN RELATION TO A WORLD MODEL

Forrester's work on world dynamics (5) and a subsequent study by Meadows et al., (7) provide startling conclusions as to the fate of the world if pollution is left unchecked. The above works are certainly open to criticism from a number of points of view, (e.g., technological advances in pollution control are omitted) but it is assumed here that the results of the models are valid in order to emphasize the role of monitoring on a world scale. Further, it is suggested that similar smaller scale models may be in order.

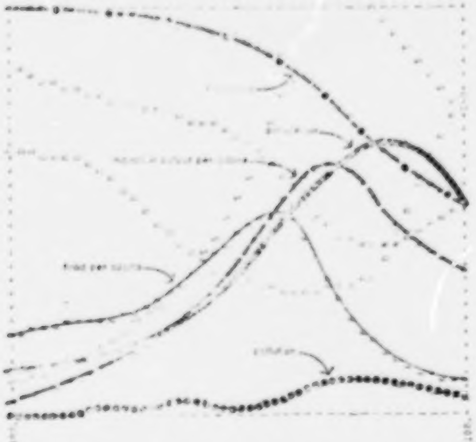
Forrester's world model is based on the assumption that the world can be described in terms of population, capital investment, pollution, agriculture and natural resources and that these variables can be described by sets of input data. Salerno (9) found the model very sensitive to input assumptions with the result that: either the world is indeed sensitive to small changes in the input descriptors; or the model is basically sound but requires more accurate input data; or the model has serious flaws. The latter view is echoed by Shubik (10) who considers Forrester's work as ". . . superficially attractive but nonetheless dangerously wrong..."

The model of Meadows et al., (op. cit.) is based on Forrester's work. The authors suggest that "...it is the only formal model in existence that is truly global in scope, that has a time horizon longer than thirty years, and that includes important variables such as population, food production, and pollution, not as independent entities, but as dynamically interacting elements, as they are in the real world." Four examples of the model output are given in Figure 2. Results are given from 1900 - 2100; the captions are self explanatory. It can be seen that the onset of "pollution" can occur quite rapidly given the assumptions and constraints of the model. The fact that complex feedback-feedforward mechanisms exist is clear from the examples. Figure 2 shows that while initial regulation and control of pollution and other factors provide for a stable population, there is an inevitable decline in industrial growth, resources and food production as pollution accumulates.

OCEAN TIME SCALES

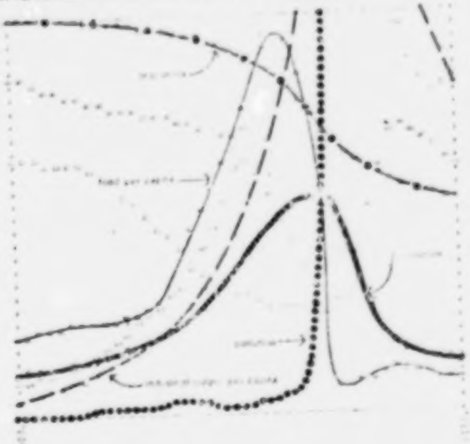
Geochemists frequently use reservoir or box models of entire oceans to investigate oceanic circulation, chemical processes within the ocean and chemical exchange at oceanic boundaries (6). As there is interchange between estuaries

WORLD MODEL WITH "UNLIMITED"
RESOURCES AND POLLUTION CONTROLS



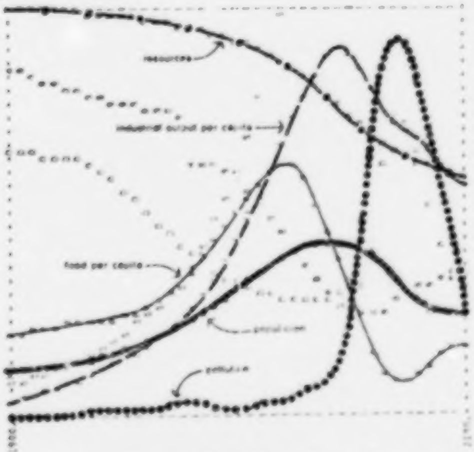
A further technological improvement is added to the world model in 1975 to avoid the resource depletion and pollution problems of previous model runs. Here we assume that pollution generation per unit of industrial and agricultural output can be reduced to one-fourth of its 1970 value. Resource policies are the same as those in figure 37. These changes allow population and industry to grow until the limit of arable land is reached. Food per capita declines, and industrial growth is also slowed as capital is diverted to food production.

WORLD MODEL WITH "UNLIMITED"
RESOURCES, POLLUTION CONTROLS, AND
INCREASED AGRICULTURAL PRODUCTIVITY



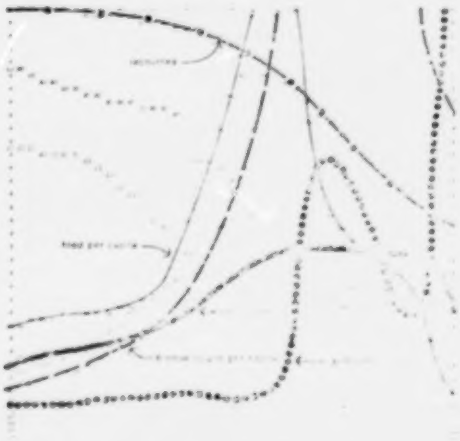
To avoid the food crisis of the previous model run, average land yield is doubled in 1975 in addition to the pollution and resource policies of previous figures. The combination of these three policies removes so many constraints to growth that population and industry reach very high levels. Although each unit of industrial production generates much less pollution, total production rises enough to create a pollution crisis that brings an end to growth.

WORLD MODEL WITH "UNLIMITED"
RESOURCES, POLLUTION CONTROLS, AND "PERFECT"
BIRTH CONTROL



Instead of an increase in food production, an increase in birth control effectiveness is tested as a policy to avert the food problem. Since the birth control is voluntary and does not involve any value changes, population continues to grow, but more slowly than it did in figure 39. Nevertheless, the food crisis is postponed for only a decade or two.

WORLD MODEL WITH "UNLIMITED"
RESOURCES, POLLUTION CONTROLS, INCREASED
AGRICULTURAL PRODUCTIVITY, AND "PERFECT"
BIRTH CONTROL



Four simultaneous technological policies are introduced in the world model in an attempt to avoid the growth-and-collapse behavior of previous runs. Resources are fully exploited, and 75 percent of those used are recycled. Pollution generation is reduced to one-fourth of its 1970 value. Land yields are doubled, and effective methods of birth control are made available to the world population. The result is a temporary achievement of a constant population with a world average income per capita that reaches nearly the present US level. Finally, though industrial growth is halted, and the death rate rises as resources are depleted, pollution accumulates, and food production declines.

Figure 2. World Model Examples
(Meadows et al., 1972)

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and coastal waters and coastal and oceanic waters, so may these interchanges be viewed as local, regional and global environments requiring equivalently scaled solutions.

Exchange of gases may occur at the air-sea interface and through reaction within the water column. In the case of particulates or nutrient balances, changes may occur at the water-sediment interface within the water column (box). If exchange rates are known, then useful information can be obtained as to the time needed to approach steady-state from given initial conditions. Ocean estimates of geochemical processes can then be made. The time frame of these processes is on the order of decades or centuries. For this simple model advection between water masses is not allowed; additional boxes would be needed with advection specified as an exchange rate.

Within a "global" box two or more regions are inferred. These may be thought of as individual boxes or connecting boxes as space or time dependent states approaching equilibirum by inclusion of additional physical processes or more detailed constituent mechanisms. Here the time scale is on the order of a day to a month.

The regional problem can itself be decomposed into a local situation where individual water parcels are traced. This decomposition may be sufficiently described by rate equations (for chemical parameters). The time scale here may be on the order of seconds to hours to days.

Of course, one can decompose the local problem even further or synthesize the one ocean model into a global model.

The system way of looking at things emphasizes that the processes involved in a given case require consideration of space and time scales.

As an example of the time involved to approach steady-state Figure 3 is presented. Here the time required to achieve 95 percent of the concentration difference between a new and initial steady-state oxygen profile is $C-C_{t=0} = 0.95\ (C_{t=\infty} - C_{t=0})$. The solution is given in terms of the physical processes of advection and diffusion versus time in years for a given consumption rate and is a solution of the equation:

$$\frac{\partial C}{\partial t} = K_z \frac{\partial^2 C}{\partial z^2} - U \frac{\partial C}{\partial z} - \upsilon$$

where

C = concentration
K = vertical eddy diffusivity
U = vertical velocity
υ = oxygen consumption rate

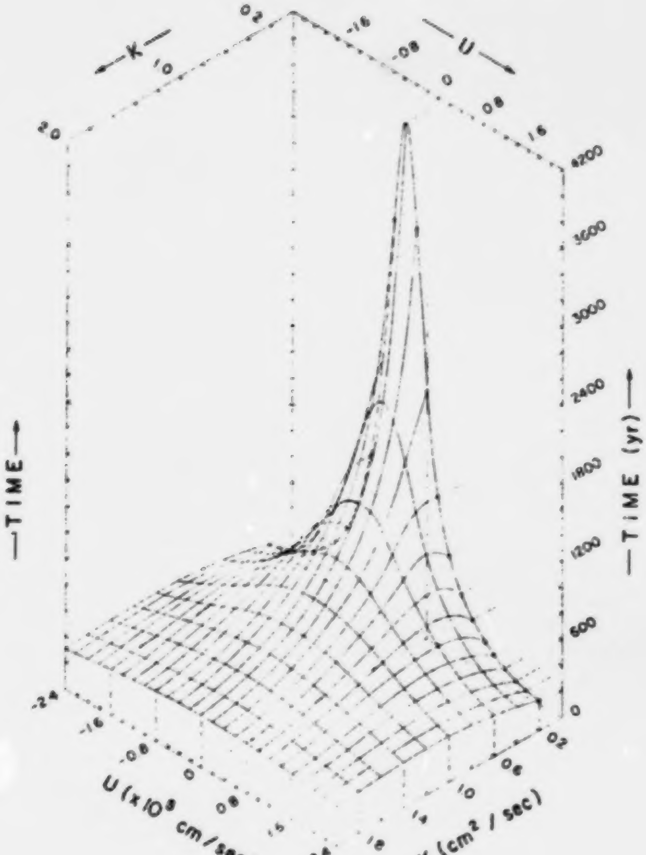


Figure 3. *Time to steady-state for oxygen concentration at $z = 1$ km*

(From Lerman, 1971)

Figure 3 shows the solution in terms of velocity and diffusion versus time. For instance, for replenishment by diffusion only (U=0), and $K_*=0.2$ cm^2 sec^{-1}, about 4000 years are required to reach steady-state. Even for more realistic values large amounts of time were still required to recover from an incident or approach a new level.

The above reasoning suggest that for some processes global monitoring is not feasible. Clearly, there are exceptions--radioactive contamination of ocean waters at depth is an example. The exceptions are usually related to non-point source inputs such as atmospheric fallout over wide areas. We should not expect, however, to routinely detect isolated point source contributions to global or oceanic pollution unless they are of extremely high input rates and high level concentrations.

Regional Time Scales

The question of what is the least sampling frequency required to monitor a trend or transient cannot be easily answered for a variety of reasons: the rate processes may vary (for nonconservative substances) depending on the type of reaction, depth, water mass, input source and strength, etc. Possibly the only truly satisfactory method would be near-continuous sampling coupled with an averaging or filtering scheme to detect shifts in water quality not obvious on a continuous data display.

As an example of the variation of rate processes to be expected, Table 1 is presented. There is illustrated a wide spread of observed and calculated oxygen consumption values (0.0027 - 5.8 ml liter^{-1} year^{-1}) for differing environmental conditions (fjords to abyssal waters). Advection and diffusion markedly affect the replenishment rate of the resource; the large values observed in fjords are due to the absence of flushing for long periods of time.

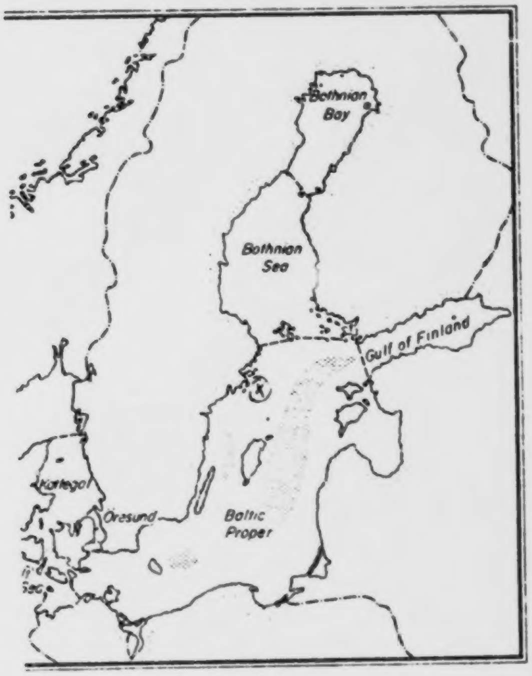
The progressively deteriorating oxygen resource and increase of phosphate levels in the Baltic below the halocline is shown in Figure 4. Data from 1900 to 1967 show considerable scatter but detectable trends on the order of about 0.1 ml/l O per year during 1957-1967 and 0.3 mg/l PO_4-P per year. It is appreciated that there is more to the interpretation of these figures than meets the eye but the example is obvious.

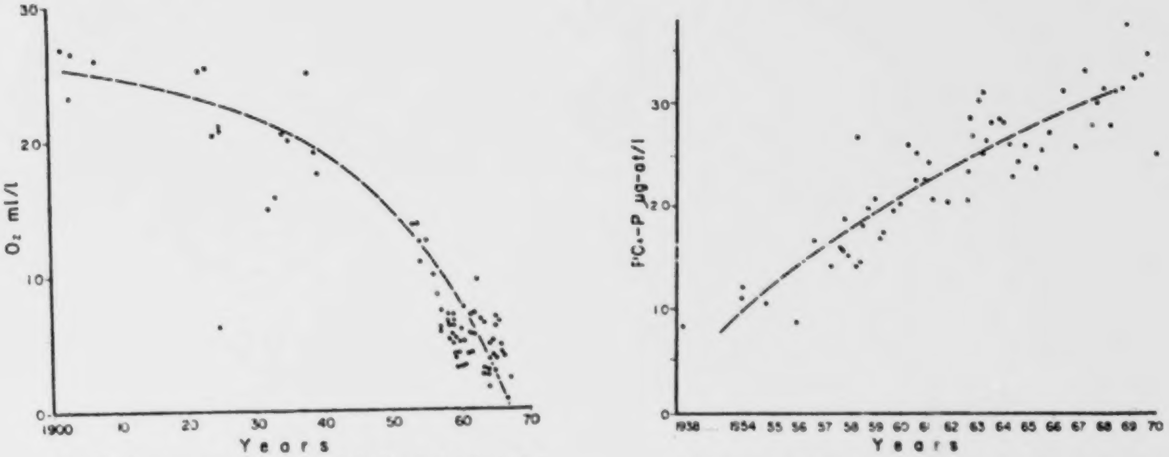
LOCAL TIME SCALES

Examples of local (order of kilometers) problems are familar to everyone and will not be discussed here except to suggest the following. While scaled down changes (from global to local) are easier to detect from one level to another lower

TABLE 1 Oxygen Consumption In Various Basins

Basin	0^2 Consumption (ml liter^{-1} yr^{-1})	Remarks	Methods	Reference
Santa Barbara	1.9 ± 0.3	May-June 1970, below 500 meters	Direct measurements	Sholkovitz & Gieskes, 1971
	0.9 ± 0.3	June-July 1970		
S. California	1.8	200 meters		
	0.6	400 meters	Advection-diffusion model	Sverdrup & Fleming, 1941
	0.05	700 meters		
G. of Maine	0.36	Stagnant pool	Measured	Redfield (in Rossby, 1936)
N. Atlantic	0.21	200 meters	Adv.-diff. model	Riley, 1951
Puget Sound	5.8	Average of several locations, fiord basins	Measured	Barnes & Collias, 1958
Tofino Inlet	4.1	Fiord	Measured	Coote, 1964
Baltic Sea	0.1	Landsort Deep (459 m) (Estimated from 1957-1967 Data)	Measured	Fonselius, 1969
Central Pacific	.0027-.0053	3000 meters	Adv.-diff. model	Munk, 1966


Bothnian Bay
Bothnian Sea
Gulf of Finland
K
Kattegat
Öresund
Baltic Proper



Dissolved oxygen content below the halocline during the period **1902-1967**

Mean values of phosphate in deep water layers **(100, 200, 300** *and* **400** *m) in the Central Baltic Sea during the years* **1938** *and* **1954-1970**

Figure 4. Baltic O_2-PO_4 Relationships
(From Fonselius, 1972)

one, there is a corresponding increase in the sampling frequency and spacing of samples to effect these observations. A few oxygen observations observed over a year will exhibit trends on the regional problem on a Baltic scale but weekly (or shorter) sampling may be required for estuarine and nearshore monitoring. These broad statements can be related to a spectrum of time scales necessary to achieve optimal monitoring operations. They will in all likelihood closely resemble the spectrum of driving forces inherent in any given environment--estuarine to oceanic.

SUMMARY AND CONCLUSION

Depending on the goals of the investigator, pollution problems can be viewed from space scales ranging from microcosmic to world. Time scales span the range from seconds to centuries. A monitoring program should seek to minimize equipment requirements, sampling frequency and the number of sampling locations. This will be determined by the type of waste input and by the physical, chemical and biological processes affecting the concentration and distribution of the pollutant. For a single parameter, instrumentation requirements, sample frequency and location may be optimized--if two or more pollutants are to be monitored it is not necessarily true that a single sampling philosophy will satisfy both needs. It is suggested that modeling may be a valuable adjunct to a monitoring program in the design stage and as a concomitant activity.

NOTE ADDED:

Boyle (3) noted that there was a typographical error in the Meadows (op. cit.) world model computer program. He stated that the error was responsible for the pollution crisis of the model and concluded that "affluence without restrictive social policies is apparently attainable." Meadows and Meadows (8) reported that the error did indeed exist but had only a small quantitative effect on the published results. The error was not responsible for the pollution crisis mode; its removal does not stabilize the model system and the conclusions are unaffected by the numerical change.

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PROBLEMS IN MEASURING TURBIDITY AS A WATER QUALITY PARAMETER

R. W. Austin*

1.0 INTRODUCTION

With the accelerated concern for the protection of our environment, including the quality of our lakes, streams, and coastal waters, we should assure ourselves that we are making maximum use of the methods available for monitoring the quality of our water resources. This does not necessarily mean a perpetuation of methods and standards generated for and borrowed from other fields. There is merit at this point in time in stepping back and taking a critical look at the state of the art of optical measurements as they are currently applied to water quality and the potential which optical measurements have for providing additional useful information.

The presently popular optical measurement for the purpose of determining water quality is turbidity. We should ask ourselves, why turbidity? What does it imply as to water quality? We have come to associate clarity of water with purity and in some respects, correctly so. In large bodies of water, as for example, lakes and oceans, we permit the adjective "blue" as an added descriptor of pure water. The implication here is that departure from these conditions means the presence of either dissolved material causing absorption or suspended material causing, primarily, scattering. By their presence these materials cause the water to lose the clear "water-white," or in large quantities, characteristic blue appearance we tend to associate with water in its purest state. Thus, green water may be due to the absorption of blue light by dissolved material such as yellow substance, or "gelbstoff," attributed to decomposition products found in the biologically productive areas of the oceans. Or the green may be due to reflectance of the chlorophyll-bearing phytoplankton found in these areas. In coastal or estuarine regions, where there may be

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significant quantities of terrigenous material suspended in the water, the lack of clarity may be due mostly to the scattering of light by the suspended silts or clays. It should be remembered, however, that these materials absorb light as well as scatter, and they do this selectively, that is, they are colored, in general, and their color varies with the type of material involved. Moreover, in estuarine waters there is frequently material in solution that absorbs light and thereby affects the appearance of the water.

Thus, the appearance of water is essentially determined by the nature of the suspended *and* dissolved material which it carries, for these materials contribute to the physical processes of scattering and absorption, both of which are involved in the subjective matter of the appearance of a hydrosol.

However, we should not be concerned solely with the subjective appearance of the water, although that may be a matter of distinct importance under some circumstances. Rather, we should seek to use the optical properties of the water as indicators of its purity or the degree of contamination by undesired material. Hopefully, by the physical measurement of some optical property or properties, we can obtain an indication of the type and amount of certain foreign materials present in a sample of water--if not on an absolute basis, at least on a relative scale.

We generally accept this lack of clarity in water as turbidity in the subjective sense. However, we do not all seem to accept the same physical definition for turbidity. Workers in colloidal chemistry, for example, carefully define turbidity as identical with the scattering coefficient alone, intentionally excluding the absorption effects from the definition (Kirk Othmer, 1969). Hodkinson, on the other hand, writing in *Aerosol Science* (Davies, 1966), defines turbidity as the product of the extinction coefficient and the path over which it is measured. Van de Hulst (1957) uses turbidity interchangeably with his extinction coefficient which is the sum of the absorption and scattering coefficients.

In the current literature of the water treatment and water quality field there also seems to be some ambiguity. In the *Water and Water Pollution Handbook*, Volume 4, turbidity is equated to the scattering coefficient alone. Yet in *Standard Methods for the Examinations of Water and Wastewater*, 13th edition, the statement is made, "Turbidity should be clearly understood to be an expression of the optical property of a sample which causes light to be scattered and absorbed rather than transmitted in straight lines through the sample."

Thus, we find in all these definitions recognition that turbidity is related to scattering, but we do not find agreement on the necessity to include the effects of absorption when measuring or defining turbidity. We will see that significant information may be neglected by excluding absorption. We will also describe some measurement techniques used in optical oceanography that might be usefully applied to coastal pollution monitoring.

2.0 VARIABILITY OF OPTICAL PARAMETERS

Figure 1 shows an Apollo IX photograph of the eastern United States coastline from about the Norfolk/Cape Henry area at the extreme north to Cape Lookout at the south, an airline distance of 142 nautical miles. Cape Hatteras is about in the center. At various points along the islands that form the barrier between Albemarle and Pamlico Sounds and the ocean, we see breaks or inlets with sediment-laden, turbid water flowing outward and along the coast. We can intuit something about the direction and relative concentration of the sediments from the reflectance pattern which the photograph presents. We should note, however, that the spatial variability points up the complexity of monitoring water quality from fixed stations. We see here only a two-dimensional representation at an instant in time. We know also that there are major vertical variations of equal or greater magnitude and that the whole three-dimensional pattern will change with tide, storms, rainfall, season, etc.

There are several points that should be emphasized. First, the reflectance of the water is a result of the combined effects of scattering and absorption: if there is no scattering, the water would appear black regardless of the absorption; if there is no absorption, scattering due to small particles or molecules would result in the blue reflectance of clean water, and scattering due to particles much larger than the wavelength of light would result in an essentially white-appearing water. It is only when both scattering and absorption are present that the water can have the full gamut of appearances.

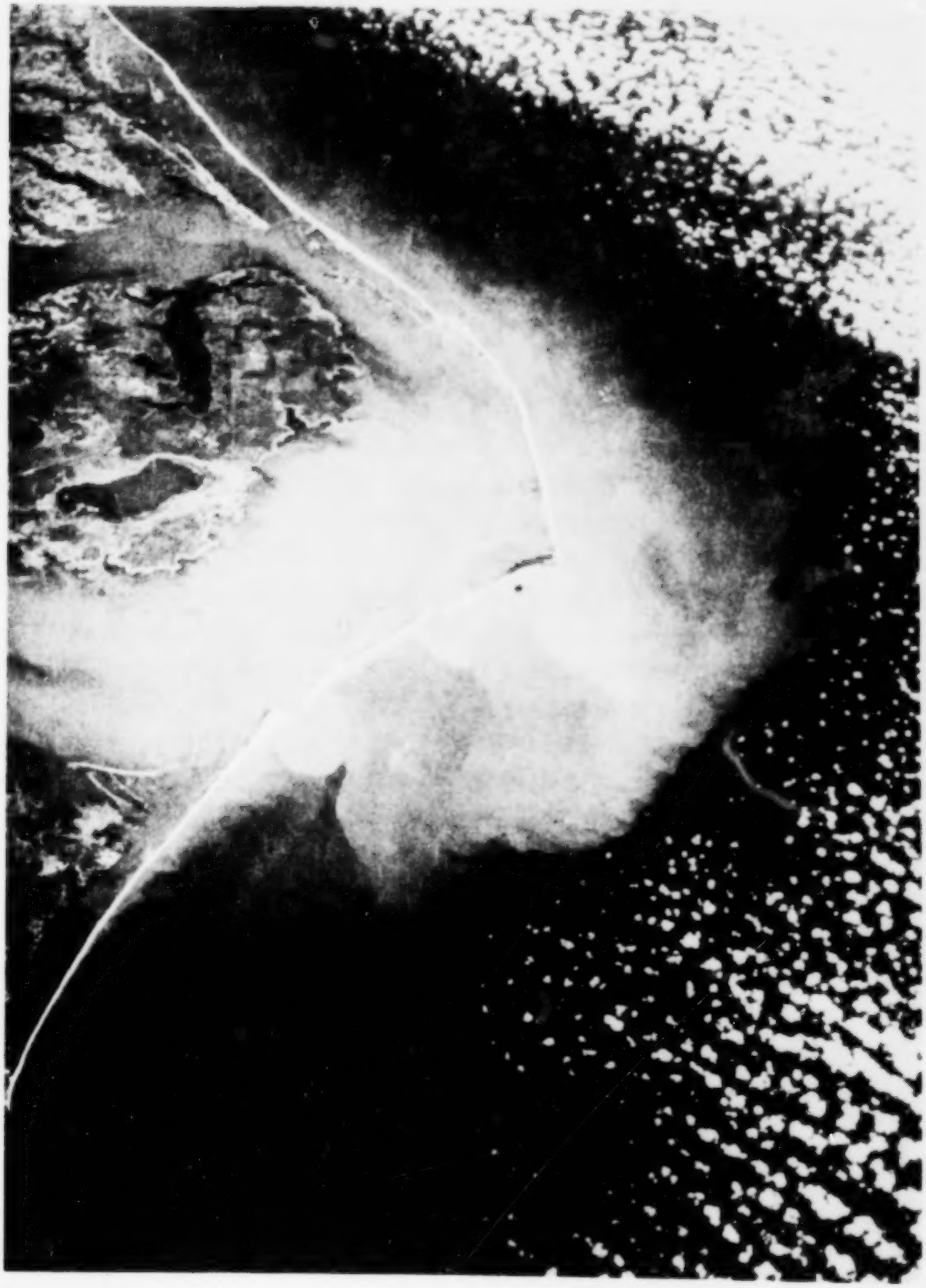


Figure 1. Apollo IX Photography of Eastern United States Coastline

A second point is that the coastal and estuarine waters are highly variable both spatially and temporally. This is a fact of nature that affects all types of monitoring of these waters, not just optical sensing. It may require a distinct change in approach for those who wish to apply the techniques common to water and waste water examination where a single measurement of a well-mixed medium may suffice.

A third point, with which most who have tried to perform observations from the deck of a surface vessel will agree, is that the degree and extent of the spatial variability is seldom apparent to the observer at the ocean surface. Thus, the selection of the number and location of sampling stations can be greatly aided if an aerial reconnaissance of the general area can be performed.

3.0 DISCUSSION

3.1 Transmittance

Let us forget about the word "turbidity" for a moment and review some of the fundamentals of light transmission through water to see how we might use the concepts.

As shown in Fig. 2, imagine a beam of essentially parallel light with a

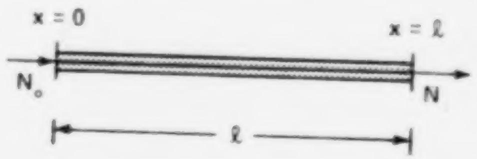


Figure 2

radiance, N_o at x = 0, passing through a medium containing both scattering and absorption. Radiance will be lost as the beam passes along a path of length ℓ in the medium. The radiance, N_ℓ, remaining at ℓ can be shown to be related to the input radiance, N_o, by the expression

$$N_\ell = N_o e^{-(a + s)} , \qquad (1)$$

where a and s are the absorption and scattering coefficients, respectively, for the medium. The transmittance, T_ℓ, for the path ℓ is simply

$$T_\ell = \frac{N_\ell}{N_o} = e^{-(a + s)\ell} \quad . \tag{2}$$

It is this transmittance that is measured by a properly designed transmissometer. We see that it is dependent on both absorption and scattering and further that we can find the magnitude of the sum of these two coefficients by taking the natural logarithm of Eq. (2). Thus,

$$a + s = \frac{1}{\ell} \ln \frac{1}{T_\ell} \quad . \tag{3}$$

Note that the dimension of these coefficients is reciprocal length. For convenience, the sum of the two coefficients is given the specific designation of volume attenuation coefficient, for which we will use the symbol α. Thus,

$$\alpha = a + s. \tag{4}$$

It should be recognized that in general, a, s, and therefore α are functions of wavelength.

Figure 3 shows a particular mechanization of the transmissometer concept which was described by Petzold and Austin (1968). It utilizes a 1-meter water path length obtained by folding the instrument at its midpoint by means of a prism. A 19-millimeter diameter projector beam falls within a slightly larger sensitive volume of the receiver whether the instrument is in air or in water. Therefore, the transmissometer may be calibrated against the air path whose transmittance is assumed to be 100 percent for 1 meter. A correction is introduced, however, to accommodate for the decrease in Fresnel reflectance losses at the four wetted surfaces when they are immersed. Thus, for this instrument the sensitivity of the receiver can be adjusted until the indicated air transmittance is 85.5 percent. With this calibration procedure an instrument embodying the proper optical design concepts will read the true transmittance of the water path.

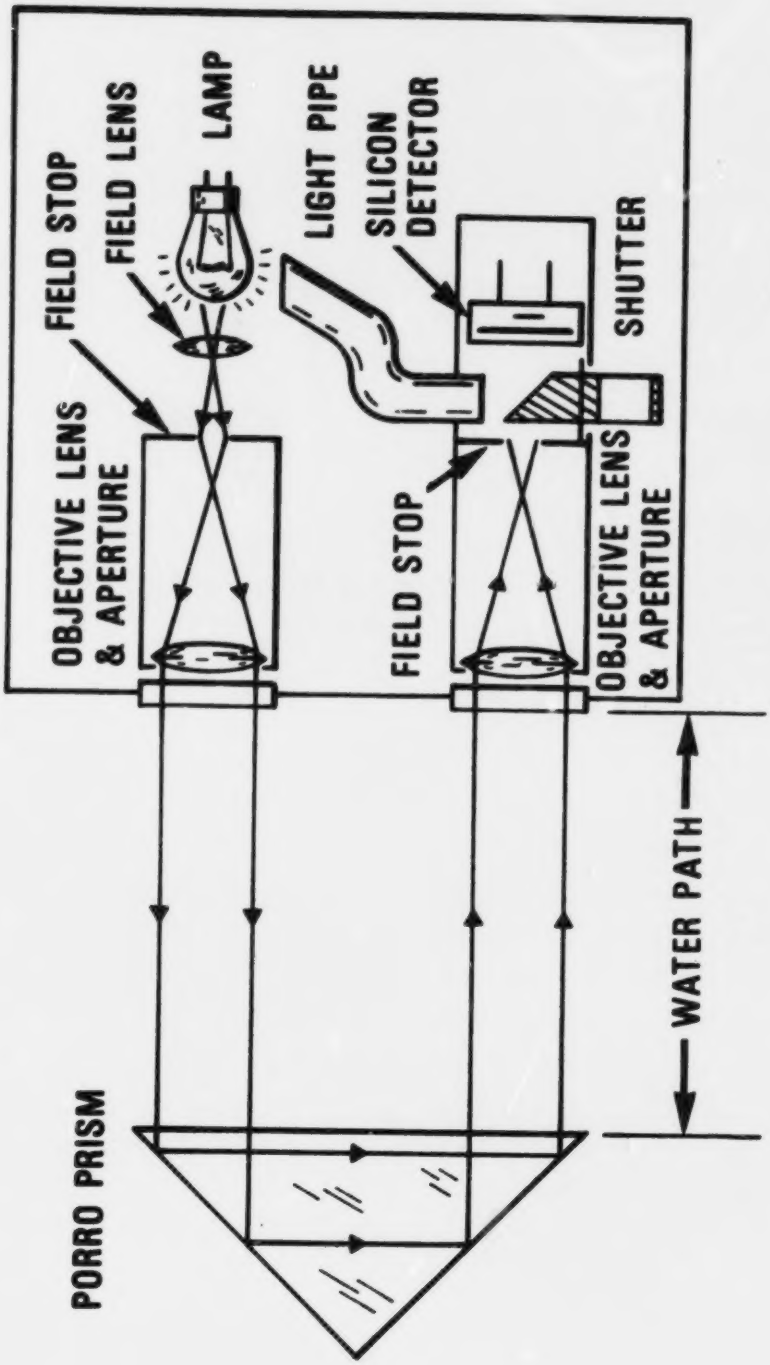

FIELD STOP
FIELD LENS
LAMP
LIGHT PIPE
SILICON DETECTOR
SHUTTER
OBJECTIVE LENS & APERTURE
FIELD STOP
OBJECTIVE LENS & APERTURE
WATER PATH
PORRO PRISM

Figure 3. Transmissometer Optical Schematic

In this instrument provision has been made for a portion of the lamp output to be directed to the detector without passing through the water path. This can be done remotely on command by the operator at any time. Thus, if a record of this reference reading is made immediately after calibration, the instrument sensitivity can be maintained at the calibration level by adjusting the receiver gain to obtain a repetition of the reference reading.

In any transmissometer design the projector and receiver beams must have finite angular dimensions. As a consequence, there is a finite amount of flux included in the measurement which has undergone scattering. Conceptually, the flux measured by the receiver should be only that which has traversed the path without being absorbed or scattered. Therefore, the scattered component which is included in the measurement represents an error. By careful attention to the design of the optical system this error may be reduced to a point where it is small compared to the temporal and spatial fluctuations encountered in most field measurements. The error present, however, will depend upon the relative amounts of scattering and absorption of the water and upon the manner in which the scattering function varies with angle. This particular instrument has been checked against other transmissometers of good design and against other methods for determining the attenuation coefficient for a large variety of water conditions. The agreement between the various determinations has been excellent, that is, within the experimental error, for most cases.

It is important in any optical measurement to be specific about the wavelength of the radiation used. This is particularly true in transmissometer measurements because of the marked change in the absorption coefficient with wavelength. The spectral passband of the instrument can be restricted very simply by the use of interference filters, by glass or gelatin absorption filters, or through the use of spectrally monochromatic sources such as lasers. The usual method for general-purpose instruments such as the one shown here is to use a combination of one or more gelatin and glass filters to obtain a moderately narrow spectral passband. In this instrument a filter wheel is provided to allow the investigator to select the wavelength. In addition, an infrared blocking filter is used because the high infrared sensitivity of the silicon detector, the large output from the incandescent lamp in the near IR, and the IR leakage of most dyed gelatin filters combine to give an unwanted IR response when the instrument is calibrated in air.

Figure 4 shows an instrument system based on the concepts just described. The transmissometer unit is shown at the bottom. The cylindrical enclosure at the left contains the lamp, the detector, the projector and receiver optics, a pressure transducer for measuring instrument depth, a thermistor probe for water temperature measurement, and the necessary electronics. The porro prism for folding the beam is at the right. The underwater unit is connected to the surface by a multiple conductor cable. The deck control unit on the right provides the necessary power for operation of the lamp and electronics in the underwater unit, plus providing the various control and signal conditioning functions required for operation. The digital panel meter can display water transmittance, temperature, instrument depth, or lamp current. Signals are also provided to a two-pen, x-y recorder shown at left, on which water transmittance and temperature may be recorded simultaneously against depth.

Other versions of this instrument have been built with provisions for changing the spectral filter by remote command from the surface, for optional display and recording of attenuation coefficient in lieu of the transmittance, and for a printer connected to the digital displays to obtain a listing of the observations.

Figure 5 is an example of the type of record obtained with an instrument of the type just described. This curve sheet presents profiles of transmittance and temperature as a function of depth taken from an area a few miles south of the tip of Santa Catalina Island off the California coast. The region is near the convergence of currents flowing down the east and west sides of the island and is known to fishery biologists as an area of high biological activity. We found the chlorophyll level to be moderately high for the region, and the water color was a very definite green. The temperature profile shows a number of small inversions near the surface and one at 23 meters probably due to the mixing of the east and west slope water. Below 37 meters the temperature was constant, indicating a mixed uniform water. The transmittance profile shows

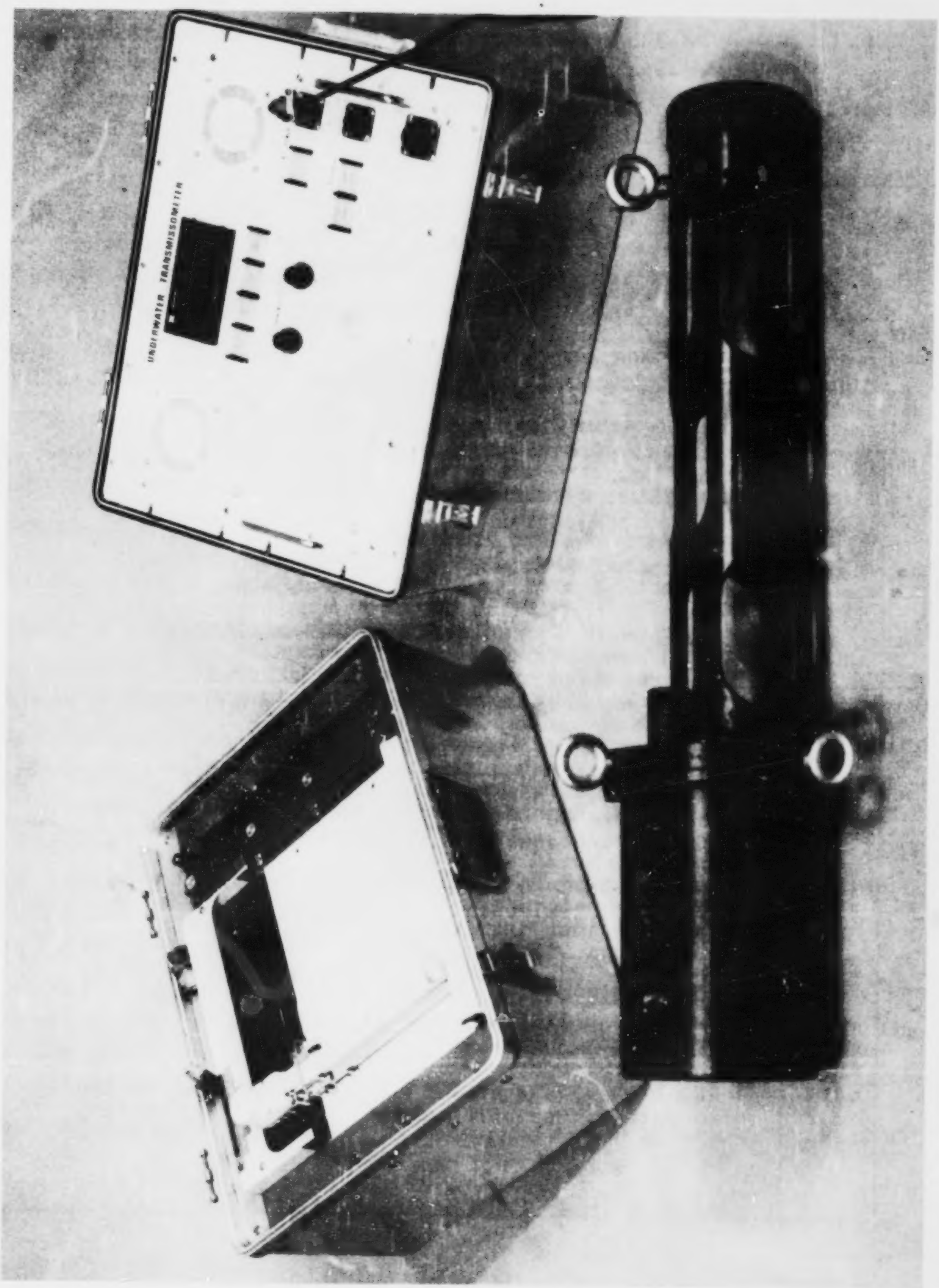

UNDERWATER TRANSMISSOMETER

Figure 4. An Underwater Transmissometer System Developed by the Visibility Laboratory of the Scripps Institution of Oceanography

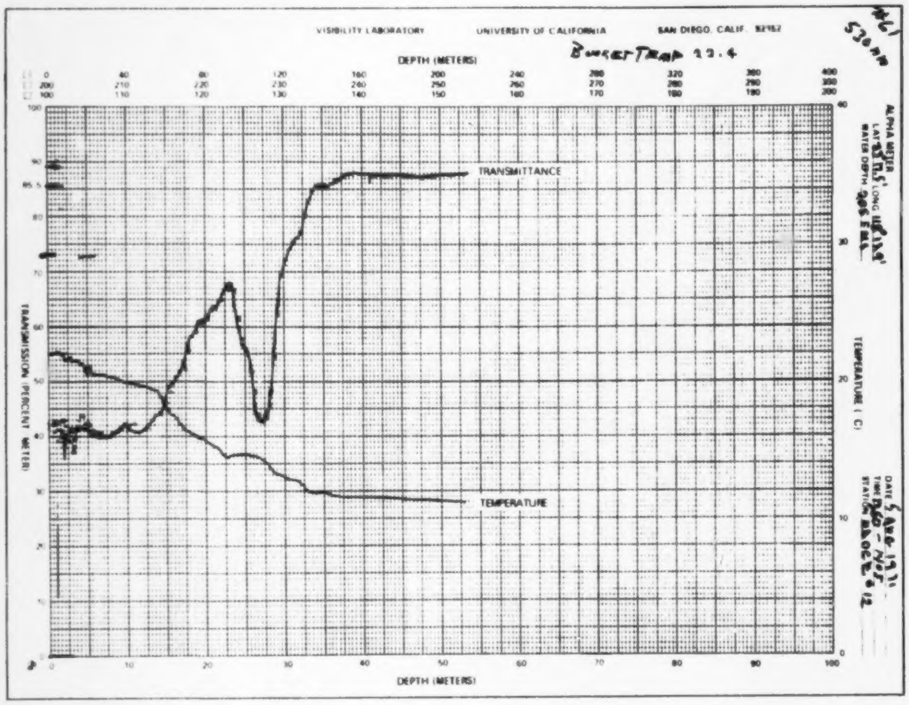


Fig. 5. Transmissometer Profile. Southern California Coastal Waters.

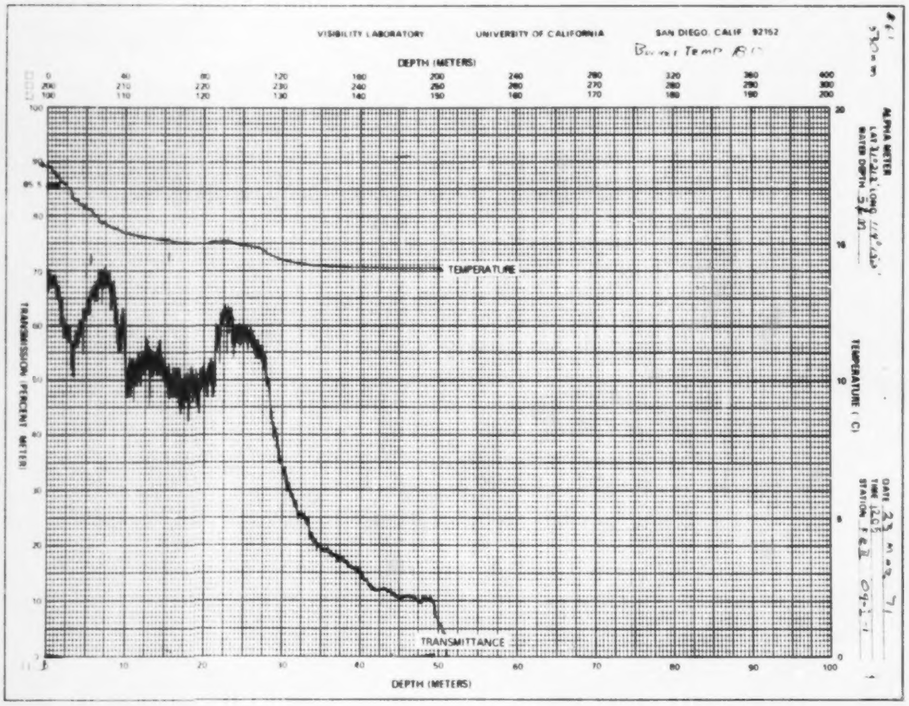
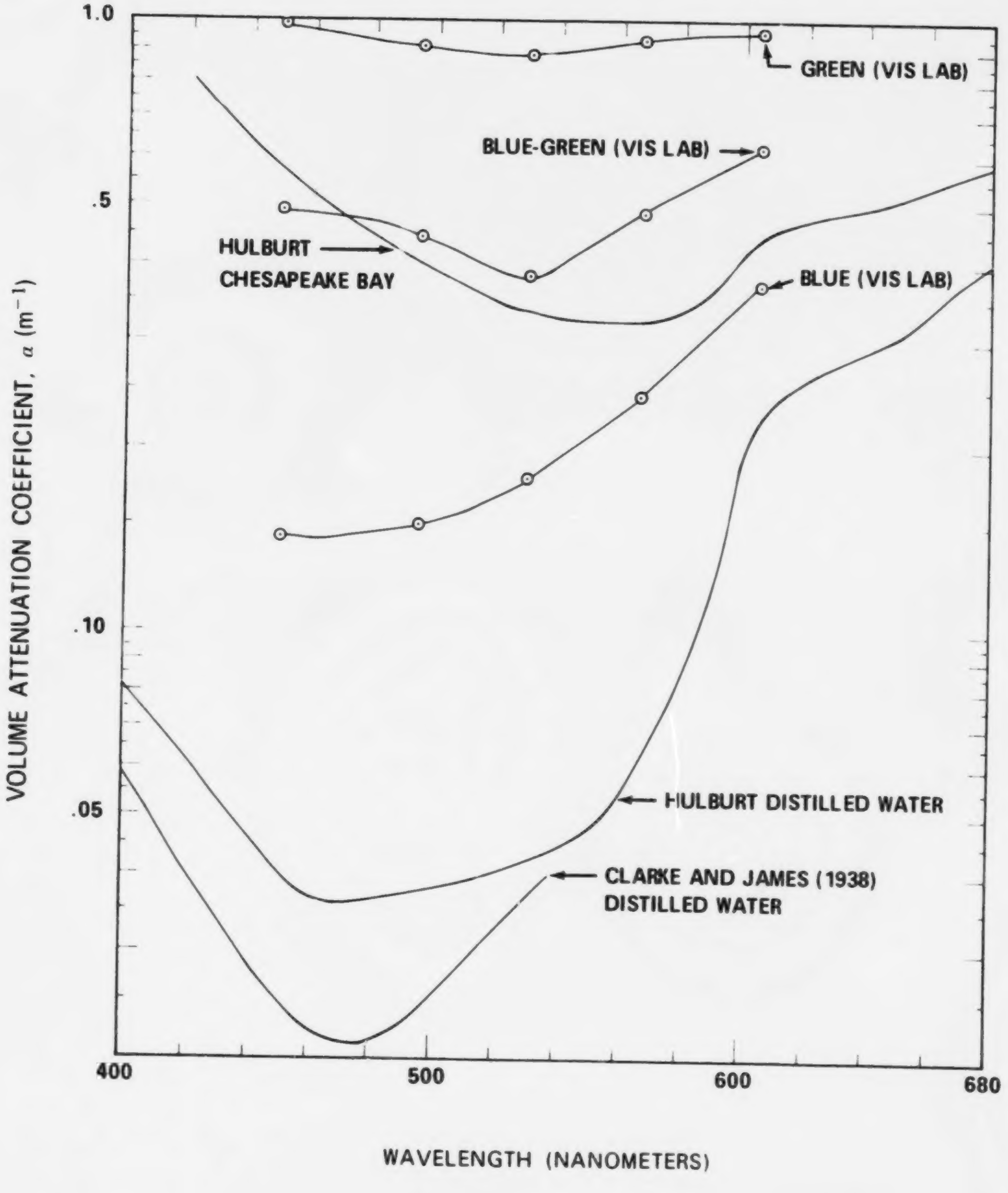


Fig. 6. Transmissometer Profile. Northern Gulf of California.



Fibure 7. Volume Attenuation Coefficient As A Function of Wavelength For Various Waters

the same features and others as well. The low transmittance water (40 percent per meter) near the surface represents a moderately turbid condition, probably a result of a large phytoplankton population with its associated high chlorophyll level and green color. At about 12 meters the transmittance started a steady increase which was interrupted by a tongue of turbid water intruding between 23 and 33 meters with a sharp minimum at 27 meters. The same feature can be seen in the temperature profile, and we might indulge in the conjecture that the combination was due to a wedge of the warmer, more turbid surface water. By 37 meters the transmittance was moderately high and uniform below this depth.

A second profile--this one taken in the northern part of the Gulf of California near the mouth of the Colorado River--is presented in Fig. 6. It shows a different structure with water of intermediate turbidity overlying heavily silted turbid water at the bottom. The noise-like trace in the 10 to 20 meter region indicates the presence of large particulates, probably zooplankton passing through the light beam. Both of these profiles were taken at a wavelength of 530 nanometers.

In the vicinity of Santa Catalina Island, measurements were made at five wavelengths on each station. The spectral changes in the attenuation coefficient computed from the transmittances in the upper 5 meters of water for three of these stations are shown in Fig. 7. The waters varied from a very turbid green productive water shown in the top curve, to the more usual blue-green coastal water in the middle curve, to the blue almost oceanic water in the middle of the graph. These may be fairly typical spectral curves for water that is neither polluted nor silt-bearing. However, we lack adequate data of this type to make definitive statements about the spectral attenuation coefficient signatures of various types of water. The spectral transmissometer, capable of rapidly shifting wavelengths, is just now becoming available as a practical field instrument. The answers it can provide may prove to be very helpful in monitoring coastal waters. It is, in fact, a kind of submersible spectrophotometer capable of providing multidimensional spectral data in situ.

For reference, a curve of the attenuation coefficient for a sample of Chesapeake Bay water reported by Hulburt (1945) is shown near the top of the

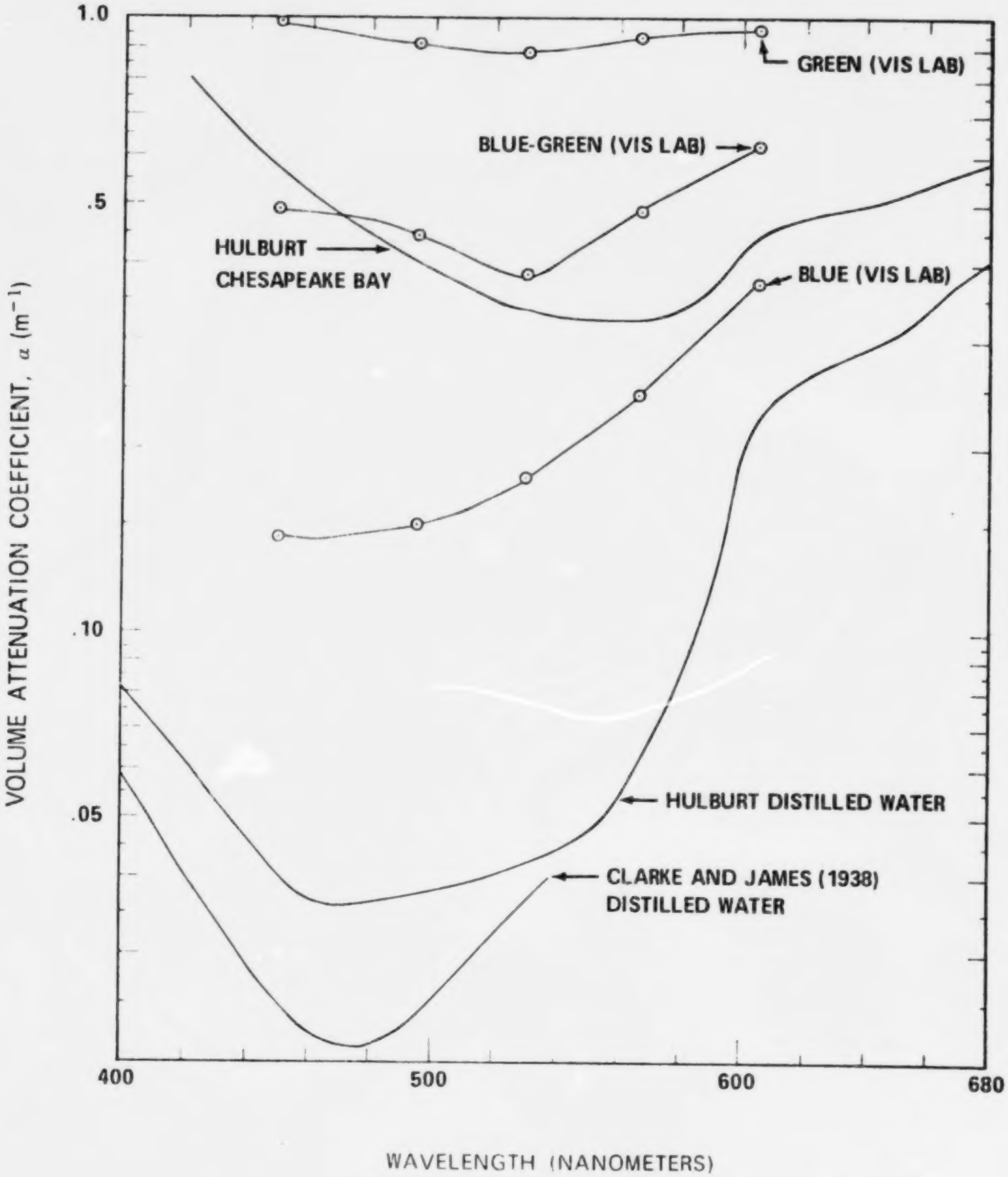


Fig. 7. Volume Attenuation Coefficient As a Function of Wavelength for Various Waters.

graph. These measurements were performed in the laboratory a day after obtaining the sample. The difference in shape may be attributed in part to the greater spectral resolution used in the laboratory measurement and perhaps due in part to changes in the sample after removal from the bay. There is also the distinct possibility that the steeper upward slope for the shorter wavelengths is due to the greater concentrations of dissolved organic decomposition products found in the bay than were present in the coastal water. The rise in the curve between 580 and 600 nanometers is an absorption band of water found in most measurements if the spectral resolution and sensitivity are adequate.

The lower curve attributed to Hulburt is based on carefully distilled water and represents one of several attempts by various investigators to achieve a measure of the attenuation coefficient for pure water. The approximate lower limit of attenuation that has been measured in the spectral region of maximum transparency, attributed to Clarke and James (1939), is shown at the very bottom of the figure. Recent measurements at the Visibility Laboratory using laser light in highly filtered fresh water have given results close to the lower curve.

3.2 SCATTERING

A common measurement technique today in water quality monitoring is nephelometry. As usually practiced, the scattering from a sample is measured at a single angle or small range of angles. Unfortunately, the common practice for the calibration of these instruments is to use an arbitrary scale devised originally for a particular form of extinction photometer known as the candle turbidimeter, or more specifically, the Jackson candle turbidimeter. The units are called "parts per million turbidity, silica scale," or simply, Jackson Turbidity Units (JTU's). The calibration resides in the form of a table of distances in centimeters versus turbidity units. Table I, taken from Standard Methods, is this calibration. The distances are the depths of the turbid water Jackson found were required for the image of the flame of a standard candle to be lost in the glow field caused by the flame's forward scattered light when

Table 1

GRADUATION OF CANDLE TURBIDIMETER†

Light Path* cm	Turbidity Units	Light Path* cm	Turbidity Units
2.3	1000	11.4	190
2.6	900	12.0	180
2.9	800	12.7	170
3.2	700	13.5	160
3.5	650	14.4	150
3.8	600	15.4	140
4.1	550	16.6	130
4.5	500	18.0	120
4.9	450	19.6	110
5.5	400	21.5	100
5.6	390	22.6	95
5.8	380	23.8	90
5.9	370	25.1	85
6.1	360	26.5	80
6.3	350	28.1	75
6.4	340	29.8	70
6.6	330	31.8	65
6.8	320	34.1	60
7.0	310	36.7	55
7.3	300	39.8	50
7.5	290	43.5	45
7.8	280	48.1	40
8.1	270	54.0	35
8.4	260	61.8	30
8.7	250	72.9	25
9.1	240		
9.5	230		
9.9	220		
10.3	210		
10.8	200		

* Measured from inside bottom of glass tube.

† Standard Methods for the Examination of Water and Waste Water. Thirteenth Edition.

viewed through the particular silica suspension he used. Needless to say, it has been difficult to reproduce his results with other suspensions, so that the calibration standard today is actually the table shown here. If one has an instrument of the design Jackson prescribed, the turbidity for any arbitrary suspension may be found in JTU's by determining the extinction distance and referring to the table. The numbers refer only to the equivalent parts per million of Jackson's silica suspensions and not to any standard physical unit. The particular silica suspension used originally is probably of no interest to present-day problems. But the unit or its derivatives persist.

In 1926 Kingsbury and Clarke developed a suspension called Formazin which could be prepared in accordance with their formulation with a reproducibility of ±1 percent of the candle turbidimeter reading. Formazin is presently used as a calibration agent for various types of turbidity measuring instruments, and the current trend is to call the unit the Formazin Turbidity Unit or FTU. Nevertheless, it should be recognized that the standard still goes back to the candle turbidimeter using the calibration given in the table for parts per million silica and not, for example, parts per million Formazin.

It is a well-recognized fact that instruments of different optical design when calibrated against the same turbidity standard may yield widely disparate answers if used to measure a sample containing different turbidity-generating material. The basic problem is that the physical principles invoked in the extinction of the light source are not the same as those used in the single angle scattering measurement.

This is not to say that useful information cannot be obtained from scattering measurements or nephelometry. Properly designed instruments calibrated in well-defined and accepted physical units can be built and may provide useful data for water quality monitoring. The techniques would be in concert with those used in optical oceanography, and the data could be compared between investigators using different instrumentation and with the existing and growing body of oceanographic data.

The basic scattering measurement may be conceptualized with the aid of the following development. See Fig. 8. Imagine an irradiance, H_o, incident

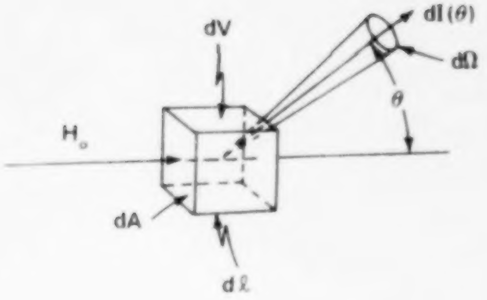


Figure 8

on a small volume, dV, which, for the moment, we will imagine has a projected area, dA, normal to the direction of propagation, and a length dℓ along the direction of propagation. If the volume contains a scattering medium and is viewed at some distance great compared to its dimensions, it will have an intensity, dI(Θ), proportional to the incident irradiance and the volume. The constant of proportionality is called the volume scattering function or VSF, here denoted σ(Θ). Thus,

$$dI(\Theta) = \sigma(\Theta) \cdot H_o \cdot dV \tag{5}$$

or

$$\sigma(\Theta) = \frac{1}{H_o} \cdot \frac{dI(\Theta)}{dV} \quad . \tag{6}$$

We can rearrange the terms and obtain

$$\sigma(\Theta) = \frac{1}{H_o dA} \cdot \frac{d\Omega \cdot dI(\Theta)}{d\Omega \cdot d\ell} \quad . \tag{7}$$

We see that $H_o dA$ is the power incident on the volume and $dI(\Theta) \cdot d\Omega$ is the power leaving the volume in a solid angle $d\Omega$. If the dimensions are kept suitably small, we may write

$$\sigma(\Theta) = \frac{P_\Theta}{P_o} \cdot \frac{1}{\Delta\Omega} \quad , \tag{8}$$

which is the fraction of the power scattered from a beam of light into a small solid, Ω, in the direction Θ, per unit path length. The dimensions of σ(Θ) are reciprocal length times reciprocal solid angle (usually $\text{meter}^{-1} \times \text{steradians}^{-1}$).

If we now integrate the VSF over all angles around the volume, we obtain the volume scattering coefficient, s, which then represents the total fraction of the power removed from a beam of light by scattering in any direction, per unit path length. Thus,

$$s = 2\pi \int_0^\pi \sigma(\Theta) \sin \Theta \, d\Theta \quad . \tag{9}$$

Figure 9 shows an optical schematic of an instrument described by Petzold (1972) which measures the VSF in situ at angles from 10 to 170 degrees. The projector and receiver have rectangular beams which intersect at the center of rotation of the projector. The volume of intersection, intersection angle, projector power, and receiver angular field are all known. The output of the receiver, therefore, can be converted into σ(Θ) in absolute physical units.

In Fig. 10, six VSF curves obtained with this instrument are shown. The two additional points shown at very small angles were obtained with another instrument capable of measurements at 0.085 to 0.34 degrees from the direction of propagation. The vertical scale covers eight log cycles and, as a consequence, some of the subtleties of the scattering curves tend to be suppressed.

The integration of these volume scattering functions has been performed, and the values of the resulting scattering coefficients are given in the upper right of the figure along with measured volume attenuation coefficients for the same stations. The latter were obtained with the transmissometer described previously. In general, the VSF's have much the same shape in the forward direction from, say, 90 to 10 degrees. We find changes in the location of the scattering minimum shifting from 95 degrees for the clearest water shown here to 150 degrees for the turbid water in San Diego Harbor. In general, the magnitude and shape of σ(Θ) show the greatest variability in the backward direction.

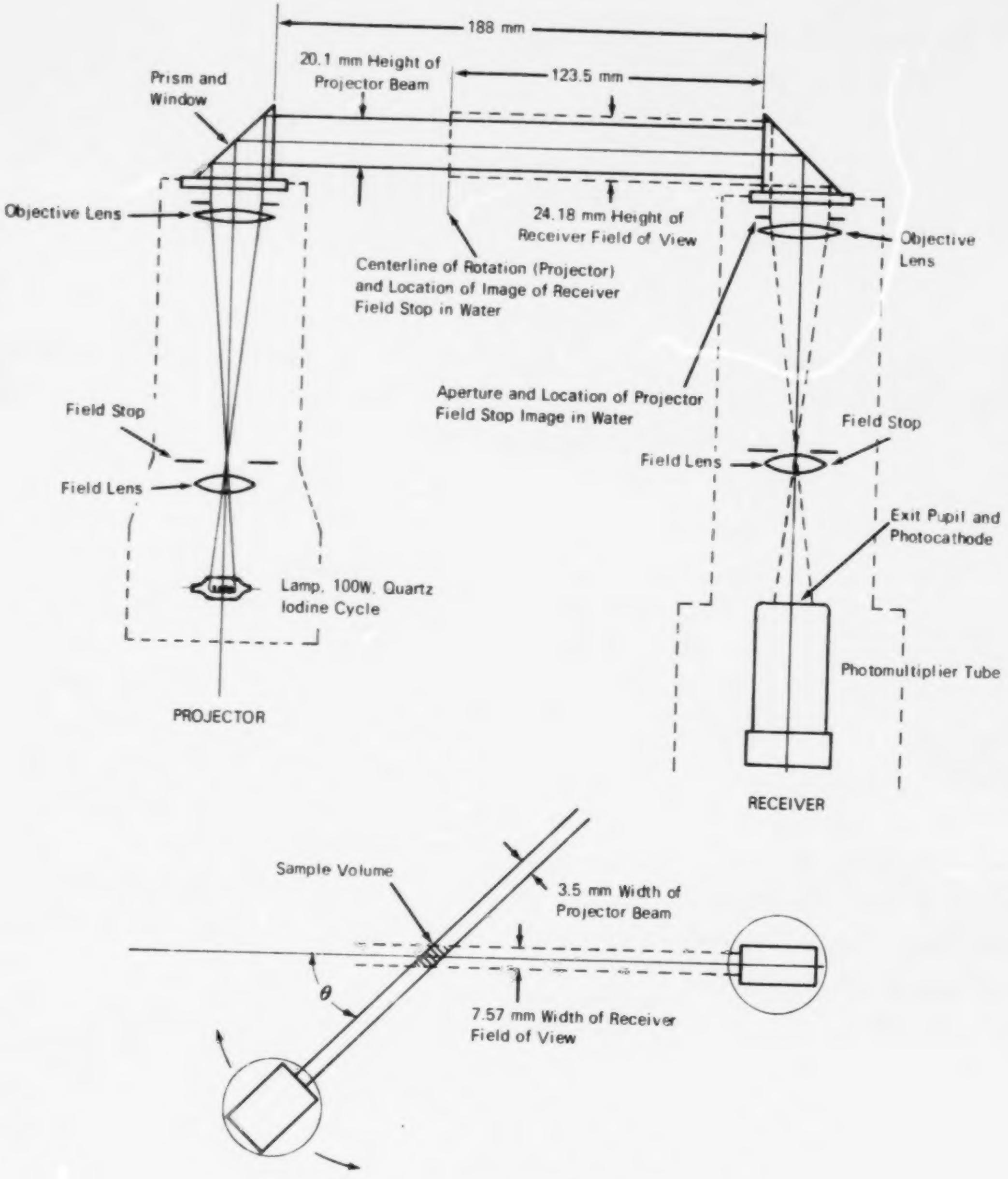

188 mm
20.1 mm Height of Projector Beam
123.5 mm
Prism and Window
Objective Lens
24.18 mm Height of Receiver Field of View
Objective Lens
Centerline of Rotation (Projector) and Location of Image of Receiver Field Stop in Water
Aperture and Location of Projector Field Stop Image in Water
Field Stop
Field Stop
Field Lens
Field Lens
Exit Pupil and Photocathode
Lamp, 100W, Quartz Iodine Cycle
Photomultiplier Tube
PROJECTOR
RECEIVER
Sample Volume
3.5 mm Width of Projector Beam
θ
7.57 mm Width of Receiver Field of View

Figure 9. General Angle Scattering Meter Optical System

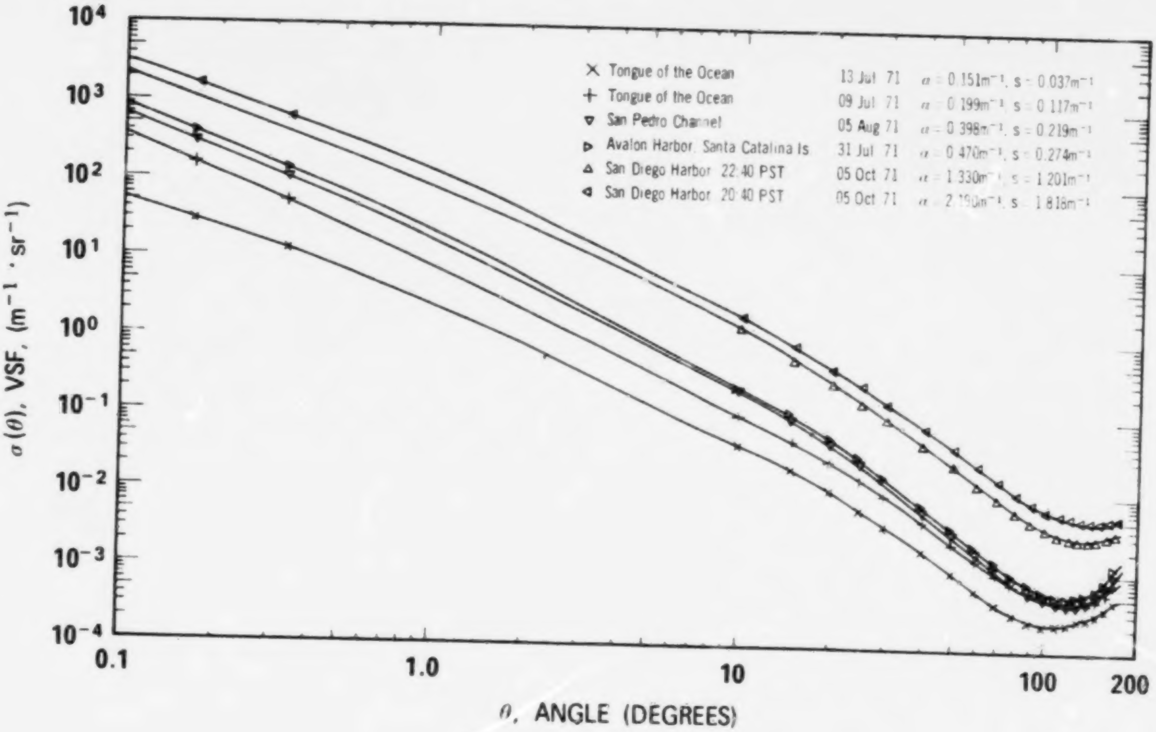


Figure 10. Volume Scattering Function of Sea Water For Various Locations

However, because the relative magnitude of $\sigma(\Theta)$ in the backward direction is very much smaller than in the forward direction, the affect of these changes on the total scattering coefficient, s, is negligible except for the clearest waters. For example, the rear hemisphere of the scattering function in water of intermediate to high turbidity contributes typically between 1.2 and 2.5 percent to the total s. The bottom curve shown in Fig. 10 for the moderately clear water in the Tongue of the Ocean has 4.5 percent of its scattering to the rear. Measurements on highly filtered water have shown values as high as 15 percent, and for perfectly clear water with only molecular scattering, the value has a theoretical limit of 50 percent.

Another indicator of the relative importance of the VSF's forward lobe is the angle required to obtain 50 percent of the total scattering, which may be called the median scattering angle. Computations performed on available data from a variety of natural and filtered waters show that this angle varies from about 5 degrees for very turbid water down to about 2 degrees for intermediate water with $s = 0.27\ m^{-1}$. It then increases again to larger angles as the water gets clearer. In the clearest water for which we have data, the median scattering angle is 19 degrees. Thus, for most coastal water the major part of the scattering coefficient results from the very narrow, 2 to 5 degrees, forward lobe of the VSF.

A corollary of this observation about the VSF is that if the object of the measurement is to obtain a number relatable to the scattering coefficient, then the measurement performed by the nephelometer should be at an angle close to the forward direction. Figure 11 shows the affect of the selection of this angle on the predictability of the total scattering coefficient. The figure shows curves for the variability of the normalized volume scattering function, $\overline{\sigma(\Theta)}$, for a range of scattering coefficients of over 100 to 1. As the normalized VSF is simply $\sigma(\Theta)/s$, it follows that if it is invariant with s, then $\sigma(\Theta)$ is proportional to s. As we see, the current practice of measuring $\sigma(\Theta)$ at 45, 90, or 135 degrees results in a large variability in the $\overline{\sigma(\Theta)}$, whereas the curve for $\overline{\sigma(5^\circ)}$ shows a much smaller dependence on the scattering coefficient.

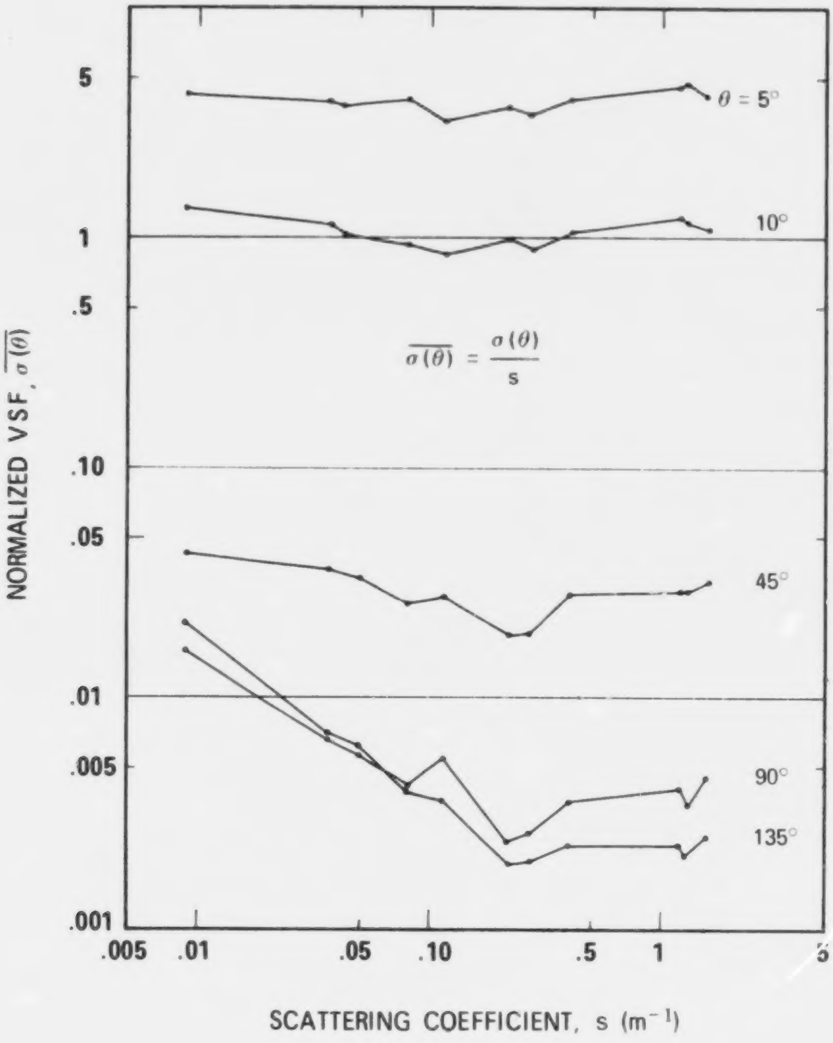


Figure 11. Normalized Volume Scattering Function $\overline{\sigma(\theta)}$ As a Function of Scattering Coefficient, s. (Note the much smaller variation of $\overline{\sigma(\theta)}$ with s for angles of 5 and 10 degrees than more conventional measurement angles fo 45, 90, and 135 degrees.)

Therefore, it is advisable to measure $\sigma(5°)$--or even $\sigma(10°)$ if the design of field-serviceable instruments would be easier and less expensive at 10 degrees--in order to obtain the best estimate of the scattering coefficient, s.

Whereas these curves were computed from data obtained by the Visibility Laboratory, other investigators have obtained the same result. Morel (1973) has made extensive Mie scattering computations for various particle-size distributions and refractive indices, and he finds that the optimum angle is from 4 to 6 degrees. These scattering data which have been attributed to the Visibility Laboratory have been obtained at a wavelength of about 520 nanometers. The wavelength for the total scattering coefficient depends on the particle sizes involved. For pure water with only molecular scattering, the total scattering coefficient will vary inversely as the 4th power of the wavelength, and for a polydisperse system having the range of particles found in the ocean, the coefficient will vary as λ^{-1} to λ^{-2}.

3.3 THE ROLES OF SCATTERING COEFFICIENT AND VOLUME ATTENUATION COEFFICIENT

We have developed the concept of beam transmittance as a means of determining the sum of the absorption and scattering coefficients. We have called this sum the volume attenuation coefficient, α. We have discussed the measurement of the volume scattering function, $\sigma(\theta)$, and the potential which exists for deducing the total scattering coefficient, s, from such a measurement performed at an appropriately small angle. Theoretically, all values of a and s could exist in the various proportions necessary to make up the range of α found in natural waters. In actual fact, the relative amounts of scattering and absorption found in oceanic and coastal waters are somewhat restricted as we see on the alpha-scattering diagram presented in Fig. 12. Data from all measurements where simultaneous α and s were obtained have been plotted here on a scale of log s versus log α. The parallel diagonal lines represent contours of constant s/a ratio. The curves represent contours of constant absorption. The sprinkle of points indicates that for the oceanic and coastal waters represented here the scattering accounts for the major part of the change in attenuation coefficient as we go from clear water shown at the left of the

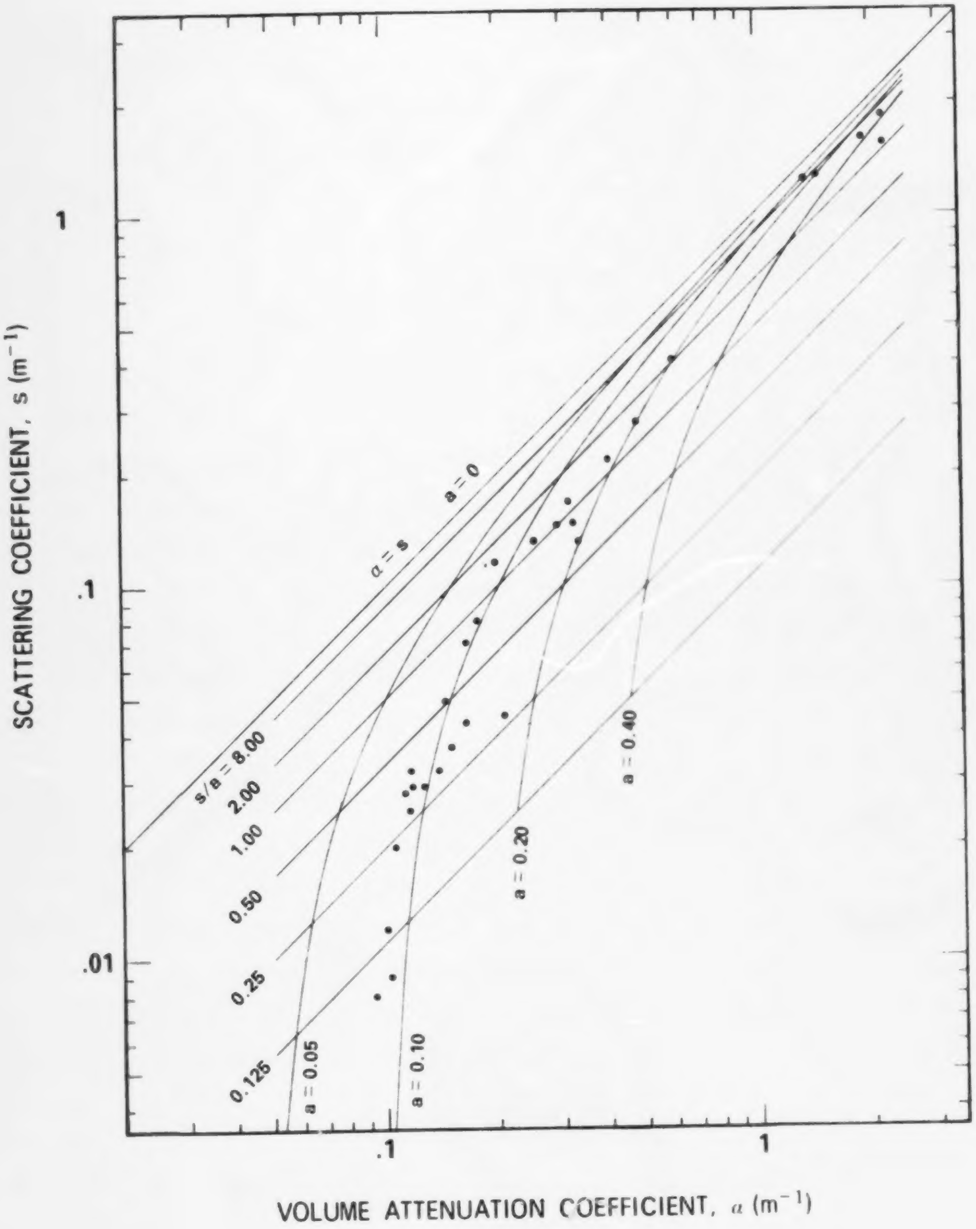


Figure 12. Plot of Scattering Coefficient Data Against Voluem Attenuation Coefficient Measured Contemporaneously For a Variety of Waters. All data obtained at a wavenlength of 520 nanometers.

diagram to the most turbid water at the right. The absorption does change somewhat, however, as α increases. These changes should not be considered insignificant. It is an artifact of this type of plot that the absorption scale is greatly compressed in the region of the large s/a values that occur when the attenuation is large. These data were obtained for a wavelength of 520 nanometers. The picture would differ somewhat for other wavelengths where the absorption coefficient has a greater or lesser affect particularly for the clearer waters.

3.4 THE RELATIONSHIP BETWEEN JTU AND α

We can attempt to relate the turbidity unit scale to the volume attenuation coefficient, α, by the following empirical method. Duntley (1971) states that, in his experiments with an underwater light source in lake water, the envelope of the lamp disappears in the glow field generated by the forward scattering from the light at about 15 attenuation lengths, i.e., $r = 15/\alpha$, where r is the distance through the water from lamp to observer at the extinction of the lamp image. Cousteau (private communication) has reported similar findings in the Mediterranean Sea. Duntley repeated his test in a tank with artificial scattering agents and found that the source disappeared at 16 attenuation lengths, possibly due to the angular scattering properties of the particular scattering agent used. In any event, we have no evidence that the threshold distance is likely to depart appreciably from the 15-attenuation length value in natural water of moderate to good clarity.

We will now apply that value to the much more turbid water for which the candle turbidimeter was designed and use the calibration values for the turbidimeter given earlier. If the JTU or concentration of silicates is proportional to the attenuation coefficient, then we might deduce from Duntley's findings that

$$(\text{JTU})k = \alpha = \frac{15}{r}$$

where k is the coefficient of porportionality and r is the threshold or extinction distance.

Note that this is a large extrapolation from the type of water in which the observations were made, however. The clearest water for which the candle turbidimeter is calibrated has an extinction distance of 72.9 centimeters (or 0.729 meter), and the corresponding attenuation coefficient would be $\alpha = 20.6 \text{ m}^{-1}$ or an attenuation length of $1/\alpha \approx 0.05$ meters! Such waters are seldom encountered except in very dirty bays and harbors or in silt-laden rivers. Conventional transmissometers with a path length of 1.0 meter are, for practical purposes, incapable of measuring a signal through such water. An instrument with a 10-centimeter path (0.1 meter) could, however, make a reasonable assessment of the transmittance of such water and even of water having a somewhat higher attenuation coefficient.

Figure 13 shows the calibration for the candle turbidimeter, i.e., turbidity units plotted against threshold distance in centimeters. The plot has been made on a log-log scale as the range of the variables extends over approximately 1.5 log cycles. On such a plot an inverse relationship between JTU and r would be a straight line with a slope of minus one. Such a line is shown (dotted) for the relationship: JTU = 2000/r (where r is in centimeters). It can be seen that the turbidimeter calibration curve has a greater slope than -1 and, in fact, appears to have a change in slope at about 30 centimeters. Thus, the simple inverse relationship does not hold precisely, although the reason does not appear obvious unless it is caused by multiple scattering. It may be that the straight line inverse relationship is a satisfactory approximation to the true extinction distance versus "parts per million silica," considering the errors and precision of the procedure.

If we use the straight line approximation shown in Fig. 13 and measure the distances in meters vice centimeters in order to have α in the more conventional units of 1/meter, then we find that

$$\alpha = 0.75 \text{ JTU } (\text{m}^{-1}).$$

We can debate whether the constant should be 1.0 or 0.5, but it is more likely to depend upon the nature of the scattering properties of the particular

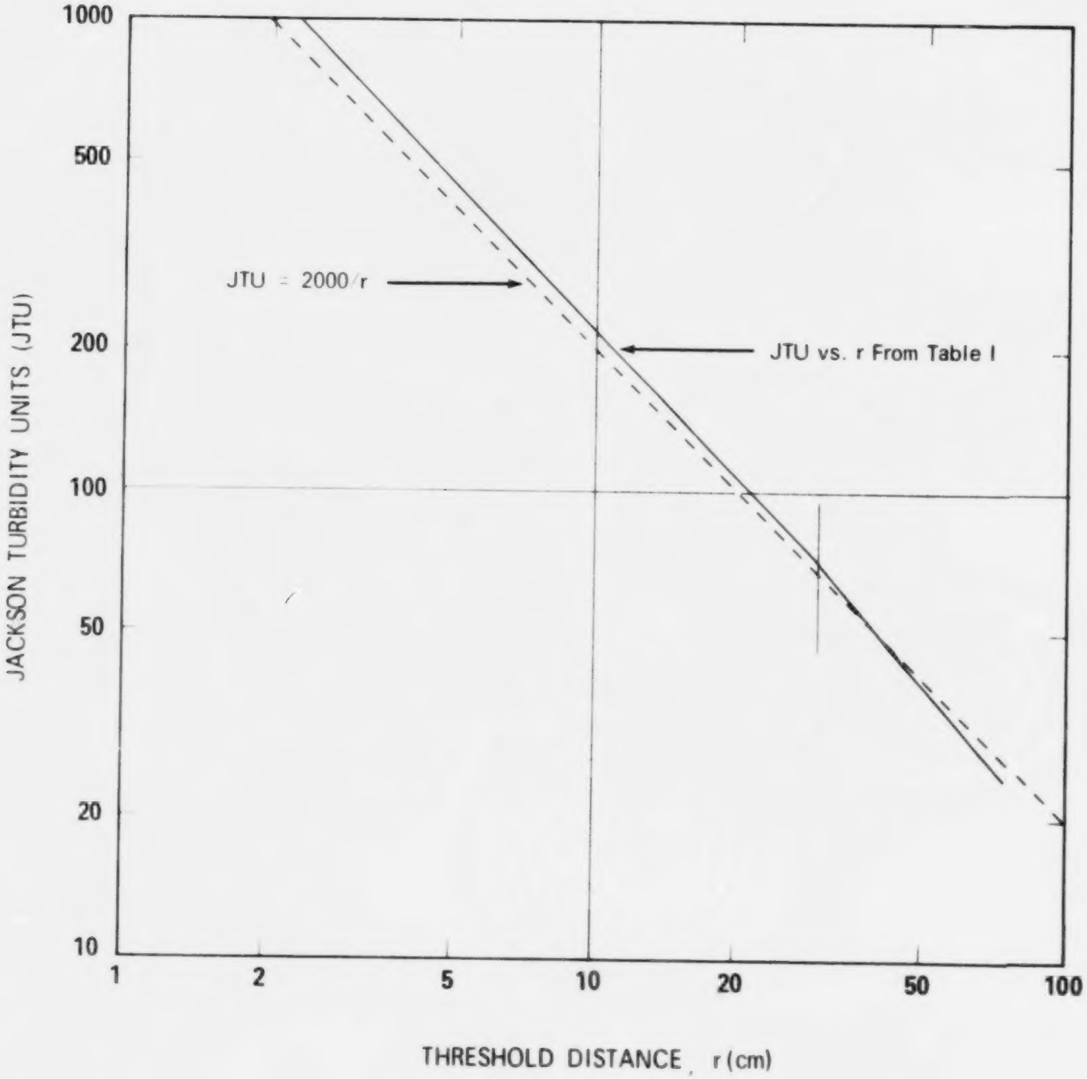


Figure 13. Plot of Jackson Turbidity Units, JTU, Against Threshold or Extinction Distance, r, in Centimeters. Solid line from Table I. Dotted line is a plot of JTU = 2000/r.

suspended material and the amount of absorbing material present than upon the niceties of any theoretical development. It is unlikely, in fact, that a single constant relating JTU and α can be applied to various suspensions apt to be encountered in the use of the candle turbidimeter.

To perpetuate the use of the JTU or FTU in measurement problems of coastal and offshore monitoring would seem, however, to be a path of dubious wisdom. The numbers so generated relate only to other data obtained with instruments of identical design and have little or no physical significance. On the other hand, instruments are available which are capable of measuring the optical variables encountered in marine waters and of providing data in units which are accepted and understood by workers in the field of optical oceanography throughout the world.

4.0 SUMMARY

Water turbidity, although due primarily to the presence of suspended material with its concomitant scattering, is also affected by absorption. The measurement of turbidity by image extinction methods such as the candle turbidimeter recognizes the effects of scattering (particularly forward scattering) and absorption. Most of the presently used nephelometric techniques for assessing turbidity in water quality work ignore the effect of absorption and make a turbidity determination proportional to the volume scattering function at some large angle (or range of angles) from the direction of propagation. In addition, these instruments are calibrated in units (JTU's or FTU's) that have little physical significance and can only be correlated to measurements made with instruments of identical optical design that have been calibrated against the same standard suspension.

Techniques and instrumentation exist for determining the volume attenuation coefficient, α, in widely accepted absolute physical units by using transmissometers of proper optical design and water path length. This attenuation coefficient is probably closely related to the extinction distances found by the candle turbidimeter but not necessarily to the JTU or FTU determinations obtained by nephelometry. These latter determinations ignore the effects of absorption and are usually made at large angles where the correlation between volume scattering function, $\sigma(\Theta)$, and total scattering coefficient, s, is poor, as shown in Fig. 11. If scattering measurements are made at around 5 degrees from the forward direction, however, the correlation between $\sigma(\Theta)$ and s is better and nephelometry can provide a good estimate of s. If a sufficiently large body of data such as that shown in Fig. 12 can be obtained for the range of variables of interest, it might lead to an empirical path from $\sigma(\Theta)$ to s to α. If this is found to be the case, a useful technique may be developed whereby small angle scattering measurements can be related to the total attenuation coefficient of the medium. For some situations scattering measurement may be preferred as when waters having a large range of turbidities must be measured with a single instrument.

For all such optical measurements, the instruments should be calibrated in accepted, absolute physical units and careful attention given to the spectral range of the radiation involved. Both absorption and scattering are wavelength dependent and the results of the mesurements and the conclusions drawn may depend upon the spectral response of the instruments used. Furthermore, a potentially significant characteristic for the study of water quality is neglected if the spectral dependence of these coefficients is not utilized.

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SURFACE SLICKS AND FILMS - A NEED FOR CONTROL

Walter F. Rittall*

INTRODUCTION

Surface slicks and films, as natural phenomena, have been under investigation for many years. LaFond (1969) has defined sea surface slicks as "...streaks or patches of relatively calm water adjacent to rippled water." This definition was adequate for his purposes but too simplistic as a definition of a potential pollutant. As research has progressed from the mechanisms of formation and movement to their physical-chemical composition, a series of results have been published that forewarn of potentially dangerous concentrating effects at the air-water interface.

PURPOSE

The purpose of this document is one of emphasis--designed to draw attention to the significance of surface accumulations. Discussions will center on one specific non-natural input area, namely that resulting from municipal waste discharges via outfalls. The regulations governing such discharges will be briefly examined to ascertain their ability to ensure adequate control and finally the discussions will turn to current research efforts.

The areas to be discussed will include work that has attempted to assess the significance of the problem. Methods applicable to field sampling, analysis of constituents and quantitative control will be put forth as candidate methods for the standardization.

THE PROBLEM

Garrett (1965) found that such surface layers are rich in fatty esters, free fatty acids, fatty alcohols and hydrocarbons - a

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finding that has been demonstrated repeatedly by subsequent investigators. Harvey (1966) in studies both in California and Hawaii found the number of algae and protozoa in a defined surface slick to be some four times the concentration at a depth of ten centimeters. This concentration factor of 4 was recently demonstrated again by Selleck (1973).

Bacteria important to the public health have been found in these surface layers, and as was demonstrated by Amies, (1956) films in swimming pool isolate the bacteria from the chlorine of the water below. Coliform concentrations as high as 1000 organisms per 100 ml have been reported for surface slicks near ocean outfalls. These counts however have been shown to be directly related to floating sewage type particles contained in the slick or film layer.

The health hazard may not be strictly aquatic. Randall and Ledbetter (1955) demonstrated that enormous quantities of nuclei containing enteric and respiratory pathogens can be transferred to the atmosphere from the surface films of activated sludge ponds. This mechanism was recently used to explain the high incidence of respiratory disease in Mexico in an area downwind of an existing sewage treatment facility. Woodcock (1955) showed that bursting air bubbles were possible mechanisms for this transfer and from the works of others it has been shown that from 0.3 to 0.7 microgram of film material accompanies each microgram of sea salt released to the atmosphere.

It has also been demonstrated by Riseborough (1968) that the microorganisms that dominate this film layer concentrate pesticides; a bioaccumulatory effect, the first of many such transfers possible through the food chain. As research continues the complexity of the problem becomes more obvious. Duce and Quinn (1972) have recently reported findings that reveal the surface layer to be an effective concentrator of trace metals, with estuarine concentrating factors* going as high as 50.

These factors help to show the general scope of the problem, however, we have completely avoided the more complex areas of carcinogenic and synergistic effects that may occur in this light available zone. These concerns are real ones and they must be considered in any monitoring program designed to be responsive to the total system. It is quite common to see emphasis in monitoring placed on the bulk water column and on one of the two interfaces that exist - namely the sediment-water interface with little or no recognition of the importance of the air-water interface.

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* The concentrating factor is defined as the ratio of the surface layer concentration to the concentration at a specified depth in the water column (often 10 cm).

REGULATIONS

Under existing federal law each state is allowed to establish its own water quality standards. The New Jersey, Alabama and California laws will be discussed here.

NEW JERSEY

New Jersey designed its own regulations to comply with the federal guidelines and adopted the following regulation for the control of surface floatable solids, oil, grease, color, turbidity and settleable solids: "None noticeable in the water or deposited along the shore or on the aquatic substrate in quantities detrimental to the natural biota. None which would render the waste unsuitable for the designated uses."

ALABAMA

Alabama's regulations, apparently as a result of noncompliance, were set by the federal government and contain only one specific reference to floating materials. This reference is contained in a definition of a minimum secondary treatment facility - "...a facility which at design flow is capable of removing substantially all floating and settleable solids..." These standards reflect an attempt to prevent the discharge from being readily apparent and it seems quite obvious that in these regulations no specific quantitative control was established.

CALIFORNIA

California has maintained its reputation for unconventional action and met the problem head on. Regulations were established that limit the hexane extractable content to levels felt to preclude surface contamination. The standard specifies that the concentration of grease and oil (hexane extractables) on the waters surface shall not exceed 10 mg/m^2 more than 50 percent of the time, nor 20 mg/m^2 more than 10 percent of the time. To cover the area of floating particulates - levels were also established that preclude concentrations of particles of wastewater origin above 1.0 mg/m^2 (dry wt) more than 50 percent of the time and 1.5 mg/m^2 more than 10 percent of the time.

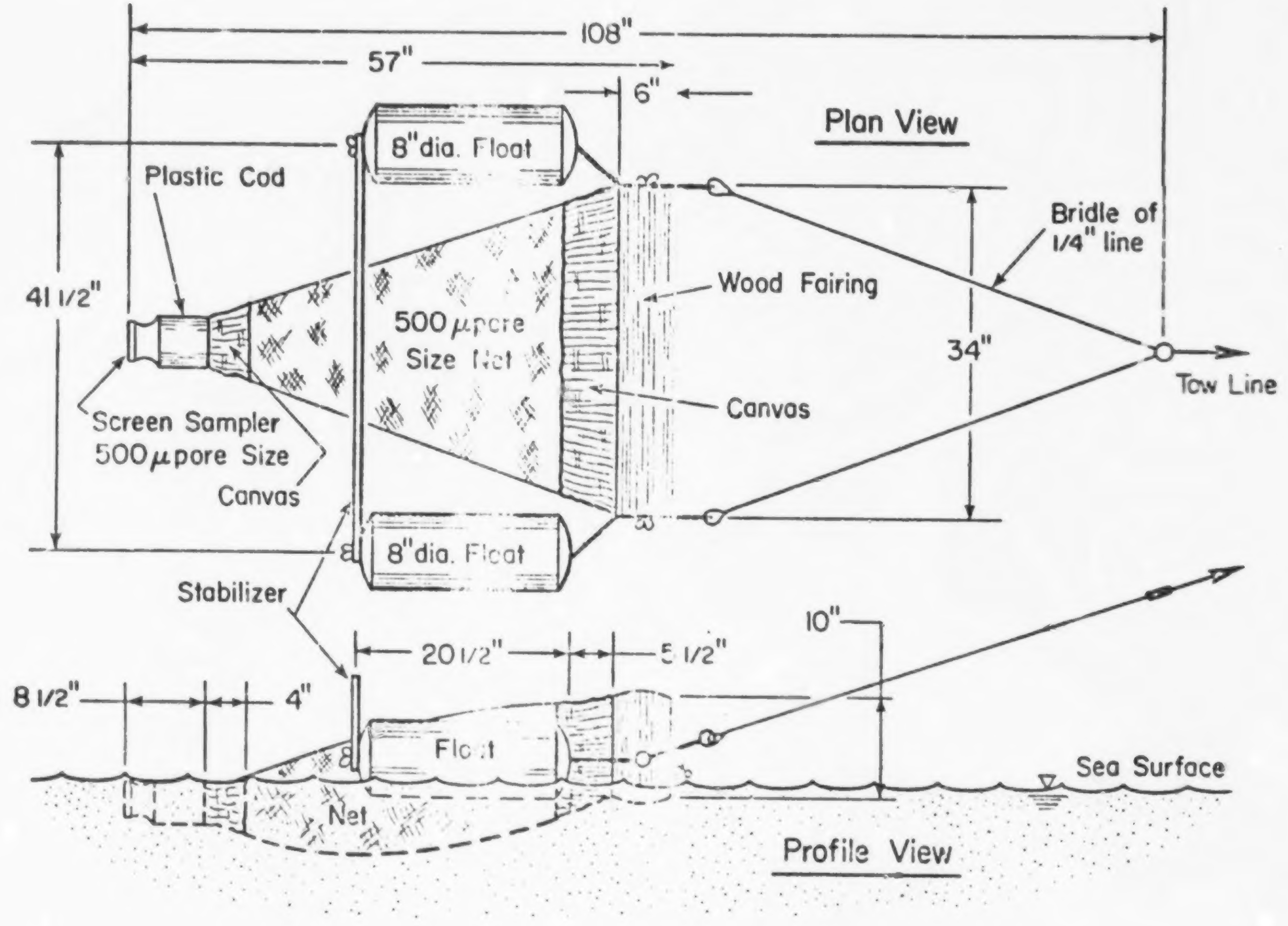
There is no doubt about the need for more quantitative regulations - there is, however, serious doubts as to the effectiveness of standards based on slick or film concentrations alone. The objective here should not be only the prevention of visible slicks, but rather the prevention of adverse concentration of any material deemed harmful to one or more segments of the total ecosystem. California has taken the first step by establishing specific limits, providing a mechanism for additions and modifications as our level of knowledge and experience grows.

OCEAN OUTFALLS

Municipal waste discharges via outfalls are not the only source of slick and film materials but they are inputs that have been studied. A stationary surface slick has been found to exist above the enormous Hyperion outfall off Los Angeles. This slick, figure 1, was visible during most sampling trips in 1971 - 1973 - trips which have coincided with the existence of strong density stratification and a submerged waste plume. This stationary slick was relatable to the waste discharge because: it assumes the same Y configuration characteristic of the diffuser; it contained a high number of floating particulates identifiable as being of sewage origin; and because it contained high levels of coliform bacteria.

How does one assess the significance of these findings? In a 1971 report to the Southern California Coastal Water Research Project (SCCWRP), Dr. Selleck, the principal investigator suggested that coliform organism die off did not occur at the rate of 90 percent in 6.5 hours as conventional techniques would indicate, but based on the inclusion of surface layer concentrations would be closer to 35 percent in 6 hours. The bacteria in the slick were associated with floating particulate material and apparently protected from normal lethal effects. These findings also suggest that the diluting and dispersive nature of outfall diffuser systems may not be nearly as efficient as the gross numbers would indicate when applied only to constituents with densities less than that of the ambient fluid.

Dilution calculations are meaningless in attempting to predict surface concentrations of surface film materials or particulates.


108"
57"
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34"
Tow Line
Canvas
Screen Sampler
500 μ pore Size
Canvas
8"dia. Float
Stabilizer
10"
20 1/2"
5 1/2"
8 1/2"
4"
Float
Sea Surface
Net
Profile View

Figure 2 TRAWL NET SAMPLER

WASTEWATER EFFLUENTS

Procedures are now being tested to try to relate the field results obtained by the above methods to the character of the effluent itself.

SUMMARY AND RECOMMENDATIONS

Experimental and field data available in the literature have shown that surface concentrations of films, slicks and particulates are important factors in effective pollution control. Ocean outfalls have been shown to be possible sources and existing water quality regulations have been questioned as to their control effectiveness.

Nonuniformity in sample collection and analyses was identified as one of the more critical areas needing attention. A need that should go hand-in-hand with the establishment of quantitative regulations. Candidate methods for the standardization of field collection procedures were presented for: surface slicks; surface bacteria; micro and floating particulates.

Two recommendations are in order. First, EPA, in compliance with current legislation, is about to publish regulations for Coastal Water Quality. They will appear for comment - in the Federal Register on October 18, 1973. Specific quantitative limits on surface concentrations are lacking and interested parties are urged to respond. Secondly, EPA's Quality Assurance Division, responsible for the standardization of procedures used in the monitoring and control of pollution, needs input from all concerned parties. Your participation will help to effectively select, test and finally standardize the more appropriate procedure.

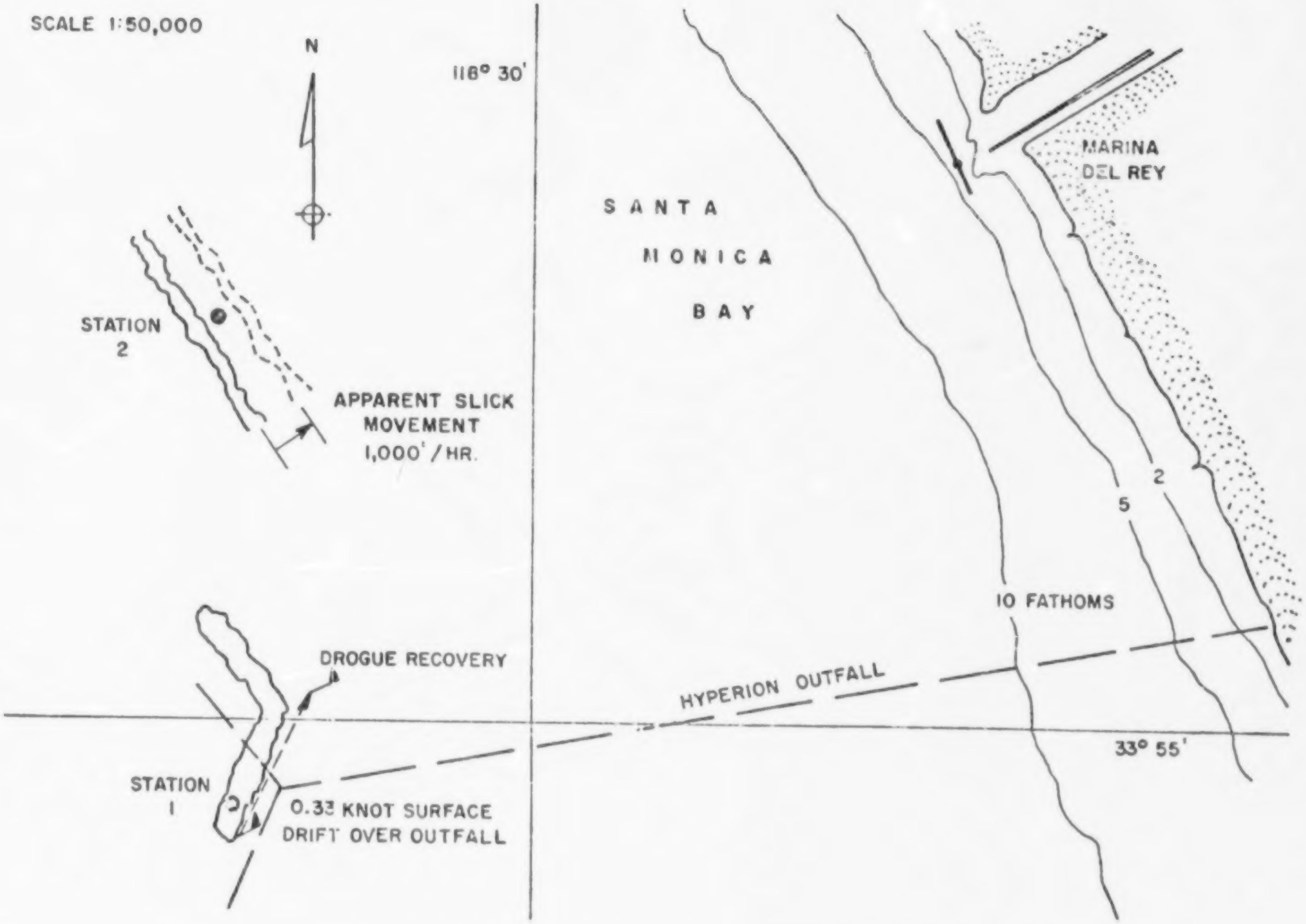
SCALE 1:50,000
N
118° 30'
SANTA MONICA BAY
MARINA DEL REY
STATION 2
APPARENT SLICK MOVEMENT 1,000'/HR.
2
5
10 FATHOMS
DROGUE RECOVERY
HYPERION OUTFALL
33° 55'
STATION 1
0.33 KNOT SURFACE DRIFT OVER OUTFALL

Figure 1 SAMPLING STATIONS 1 & 2, SANTA MONICA BAY--APRIL 7, 1971

These materials are not prevented from accumulating on the air-water interface by such intermediate phenomena. They are affected, in terms of areal concentration, by the gross size of the diffuser, the size of the waste plume, its height of rise and of course the physical-environmental parameters descriptive of the water body itself i.e., the water motion, depth, density and characteristic diffusive nature. This argument applies equally well to the bacterial distributions found associated with floating particulates.

It is unlikely that the long term accumulative nature of a continuous discharge could ever be realized in open coastal waters because of high rates of degradation and bioutilization. Physical factors such as wind and waves also contribute here acting to break up and disperse the material when certain limits are exceeded. The dispersed materials, however, do not vanish, and when the winds and waves subside they may become part of another surface slick far removed from any point source and hence be interpreted as a completely natural slick.

Surface slicks and films have been shown to be partially composed of long chain fatty acids and hydrocarbons. Farrington and Quinn (1970) investigated the concentration of these materials in treated wastewater effluents and have estimated that somewhere between 28,000 and 140,000 metric tons were discharged to coastal waters during 1970; an input that equals or betters reported accidental oil spills during the same period.

These calculations don't have to be precise to warn us of a potential danger. We are very cognizant of the acute effects of large oil spills and we know that there are chronic effects as well. The point here is simple - municipal waste discharges contain essentially the same level of hydrocarbons and must also carry with them some chronic effects. Little is known about the ability of coastal ecosystems to degrade these materials and as has been indicated simple dilution is not enough.

It would be wrong to conclude that all ocean outfalls produce stationary surface slicks since we don't know how differing degrees of treatment nor discharge method influences the net effect. It is reasonable, on the other hand, to conclude that based on field data the currently applied aesthetic-visible-restrictions have not been totally effective in the true sense of environmental protection.

SAMPLING METHODOLOGY

It is difficult to assess the true significance of surface slicks and films because of the current nonuniformity in the procedures used to collect and analyze such materials. EPA has been sponsoring research designed to fill this need through the development of basic procedures for film collection, surface bacteria collection and the collection of representative near surface samples of particulates. Analytical methods are being explored that have application to coastal monitoring schemes.

Preliminary research findings, backed by two years of effort have found the following procedures simple to apply and effective in areas both with and without visible surface films.

SURFACE SLICKS AND FILMS

The collection of the surface slick samples is accomplished with the use of a flexible screen made of glass cloth. Laboratory tests showed that such screens collected salt water by capillary action to a depth of 0.15 mm. The size of the cloth used in field applications is 0.1 m square.

The actual sampling sequence is as follows: The glass cloth is solvent washed in the laboratory to minimize the background concentration of the specific constituent to be analyzed for (in this case normally a hexane extractable one), and placed in a glass container with a teflon lid. The cloth is placed on the sea surface using a special device designed to spread the cloth and prevent contamination. The cloth is allowed to wet completely at which time it is retreived using glass rods and returned to its solvent rinsed glass container and sealed.

There is one undesirable effect from this procedure and that is the loss of water as the cloth is folded and reinserted into the container. This loss may be corrected for, but the significance of this correction is as yet unknown.

BACTERIAL SAMPLING

Nylon netting of coarse weave was used for this purpose. Laboratory studies indicated good bacterial release when the sample was washed with a sterile buffered solution. The netting used had a two filament knit with 0.75 to 1.0 mm openings and was found to collect a layer averaging about 0.1 mm at 22°C. In reducing the data the area sampled was calculated by dividing the volume of water by 0.1 mm rather than assuming that the full 0.1 m square was the true area. It is noted that the pretreatment given the cloths did cause some shrinkage. Samples were washed with 150 ml sterile buffer, capped shaken (at least 50 times) and aliquots withdrawn, filtered immediately and incubated for later counting.

MICRO PARTICULATES

A 0.55 sq m nylon net was used for this purpose. The procedure for all surface layer collections was essentially the same. This sample is refrigerated and preserved with formalin until it can be examined.

FLOATING PARTICULATES

A trawl net was used to collect floating particulates from the ocean's surface. The sampler, see Figure 2, was towed at 1.5 knots. The water entering the device is forced over a submerged airfoil and then is forced through a 500 µ removable nylon screen. The area sampled averages 87 cm wide and 3.0 cm in thickness.

ACKNOWLEDGEMENTS

The methodology and procedures discussed in this paper were developed as part of an EPA Research Grant (800373) to the University of California, Berkeley. The principal investigator was Dr. R. E. Selleck. Others working under his direction included Ralph Carter and Lloyd Bracewell, doctoral candidates at the University.

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A SYSTEMS APPROACH TO MARINE POLLUTION MONITORING

S. P. Pavlou*, T. E. Whitledge, J. C. Kelley, and J. J. Walsh

INTRODUCTION

Perturbation of ecosystem (or ecological) stability by the introduction of man-made chemical pollutants in the biosphere is a problem of international concern.

To date, the detection, identification, and measurement of biologically active pollutants in the marine environment has been accomplished in some detail (trace metals, synthetic organics, radionuclides). End effects due to biological amplification (extinction of certain species and potential toxification problems for man) have also been investigated with great concern. However, despite the steadily increasing sophistication in experimentation and the introduction of new analytical techniques in the field of pollution research, useful and predictive information concerning the response of biological systems to man-made perturbation is often empirical, descriptive, and generally obtained by scattered investigations and non-rigorous crash programs.

Marine pollution monitoring is a complex problem and requires the utilization of multidisciplinary scientific knowledge and a well organized field program for understanding the transfer of pollutants and interactive processes taking place throughout a given marine ecosystem, as well as predicting and controlling their consequences. This type of research has been initiated only recently.

We are currently engaged in international research involving evaluation of the physical, chemical, and biological dynamics of marine ecosystems within various ocean upwelling regions and assessment of biological response of marine ecosystems subjected to man-made perturbations, For the second case, while primary emphasis has been placed on eutrophic systems (Puget Sound, Southern California Bight), preliminary work has been initiated on oligotrophic systems (Eastern Mediterranean) to compare the impact of nutrient and toxic compound addition on these two distinctly different ecosystems.

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*Department of Oceanography, University of Washington

This type of multidisciplinary approach has been adopted to serve the following priorities: developing a sophisticated understanding of marine ecosystems under man-made perturbations; acquiring a predictive capacity for short and long term response of the primary productivity and fish population within a specific coastal area subjected to high pollution inputs; and assisting governmental agencies in establishing effective water quality standards.

The overall framework and a summary of the new methodology as is currently employed during our field investigations, is described below.

OVERALL FRAMEWORK

A schematic diagram showing the important processes characterizing a polluted marine ecosystem is given in Figure 1. Upon introduction of a chemical in the marine environment, the degree of interaction with the biological components of the system is a critical factor in assessing the overall impact to the ecosystem. Assuming that the physical parameters of the system (advection, diffusion, turbulent mixing and boundary conditions) are defined, the response to the perturbation depends on a number of important physiochemical factors: (1) the chemical structure of the microcomponent, (2) the reactivity with the medium (alteration of the equilibrium condition of the system's reservoir), (3) the reactivity with various constituents of the medium (chemical speciation), (4) the transport process to the biological site of interest, (5) the specific interaction on the cell wall, (6) the inhibition of enzymatic processes within the cell reflected by the alteration of the growth characteristics of the organism, and (7) the recycling and regeneration process.

These considerations have some important connotations to the monitoring methodology, as well as the construction of a simulation model of a polluted area. Quantitative definition of the above physiochemical factors and their utilization in the theoretical framework are necessary for making sound predictions about the resiliency of the system under a given pollution load. Therefore, a rigorous monitoring program should include a capability for obtaining data on the chemical inhibition of various biological rate parameters (photosythesis, nutrient uptake, enzymatic activity, grazing, etc.) characterizing the primary trophic levels of the ecosystem under investigation.

In order to satisfy these priorities, a capacity has been established for carrying out controlled experiments on

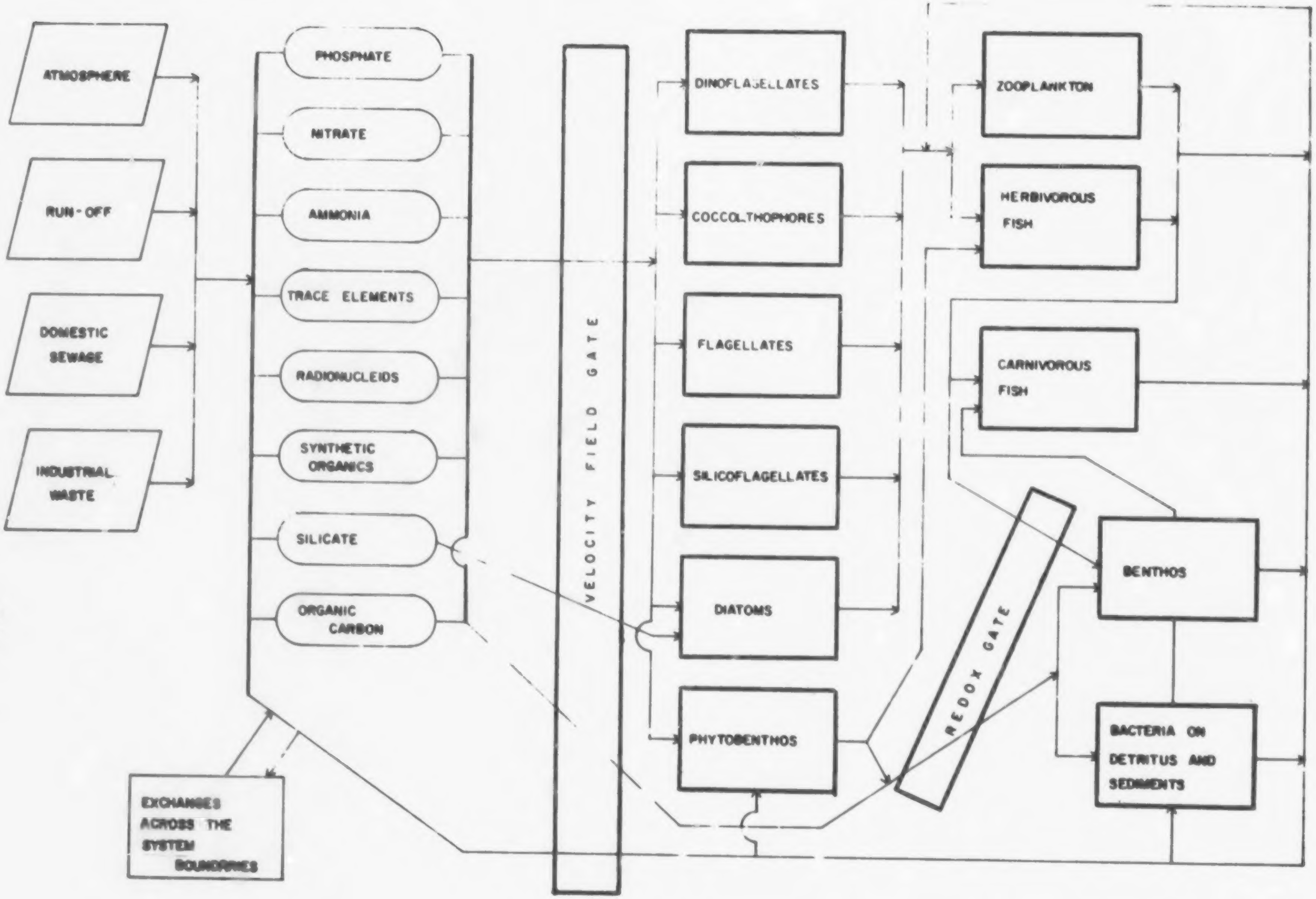

ATMOSPHERE
RUN-OFF
DOMESTIC SEWAGE
INDUSTRIAL WASTE
PHOSPHATE
NITRATE
AMMONIA
TRACE ELEMENTS
RADIONUCLEIDS
SYNTHETIC ORGANICS
SILICATE
ORGANIC CARBON
EXCHANGES ACROSS THE SYSTEM BOUNDRRIES
VELOCITY FIELD GATE
DINOFLAGELLATES
COCCOLTHOPHORES
FLAGELLATES
SILICOFLAGELLATES
DIATOMS
PHYTOBENTHOS
REDOX GATE
ZOOPLANKTON
HERBIVOROUS FISH
CARNIVOROUS FISH
BENTHOS
BACTERIA ON DETRITUS AND SEDIMENTS

Figure 1. Block Diagram of Important Processes Characterizing a Polluted Marine Ecosystem

the components of the system, describing the spatial relationships of these components, integrating the components into a general system model, predicting the behavior of the system, and testing the predictions in the field. These aspects have been approached and developed simultaneously to accomplish the proper interrelations. Together with an intense physical oceanographic program, the chemical and biological studies include the following capabilities:

1) An experimental laboratory in which the specific effects of particular limiting nutrients and chemical inhibitors on a selected species of phytoplankton can be studied under controlled conditions.

2) A shipboard data acquisition/reduction (DA/DR) and presentation facility (PF), heavily graphics oriented. A block diagram of the DA/DR system is shown in Figure 2. This facility is essentially a bank of sensors (including chemical AutoAnalyzers) and a computer capable of acquiring, editing, and analyzing data from the sensor array and presenting this data graphically as it is acquired. A data storage facility is also necessary. The DA/PF has been developed and is currently in operation in our laboratories.

3) Experimentation on nutrient, pollutant and energy fluxes through the primary trophic levels of the food chain, carried out in the field with naturally occurring organisms and ambient water.

4) A computer simulation model of a polluted marine ecosystem. This model is currently being constructed and will be used to integrate the results of the field measurements, the field and laboratory experiments, and other related information, in order to predict the behavior of the total system under specific pollution stresses. The model identifies important diagnostic features of the ecosystem which can be verified by the other three activities of the program. Utilization of the data acquisition system allows us to interpret the behavior of the system under study and assess the time dependence of its state.

OPTIMUM ANALYSIS SCHEME

The actual analysis of the area under investigation is carried out as shown in Figure 3.

1) Using the data acquisition system, continuous records of the surface distributions of the important variables (temperature, salinity, the nutrients,

Integrated Sampling and DA/DR System

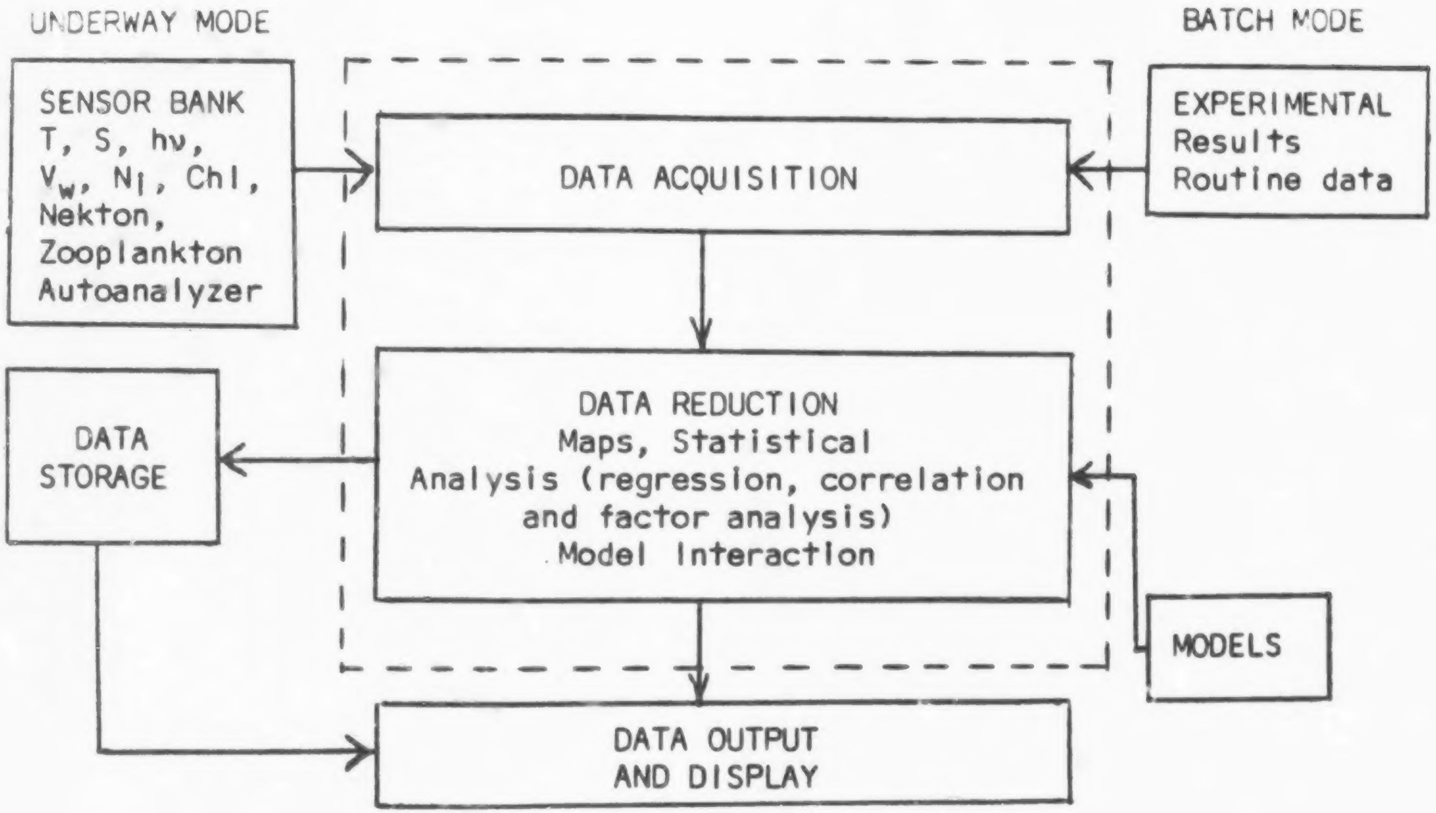

UNDERWAY MODE
SENSOR BANK
T, S, hν,
Vw, Nl, Chl,
Nekton,
Zooplankton
Autoanalyzer
BATCH MODE
EXPERIMENTAL
Results
Routine data
DATA ACQUISITION
DATA REDUCTION
Maps, Statistical
Analysis (regression, correlation
and factor analysis)
Model Interaction
MODELS
DATA STORAGE
DATA OUTPUT
AND DISPLAY

Figure 2. Integrated Sampling and DA/DR System

OPTIMUM ANALYSIS SCHEME (CYCLE)

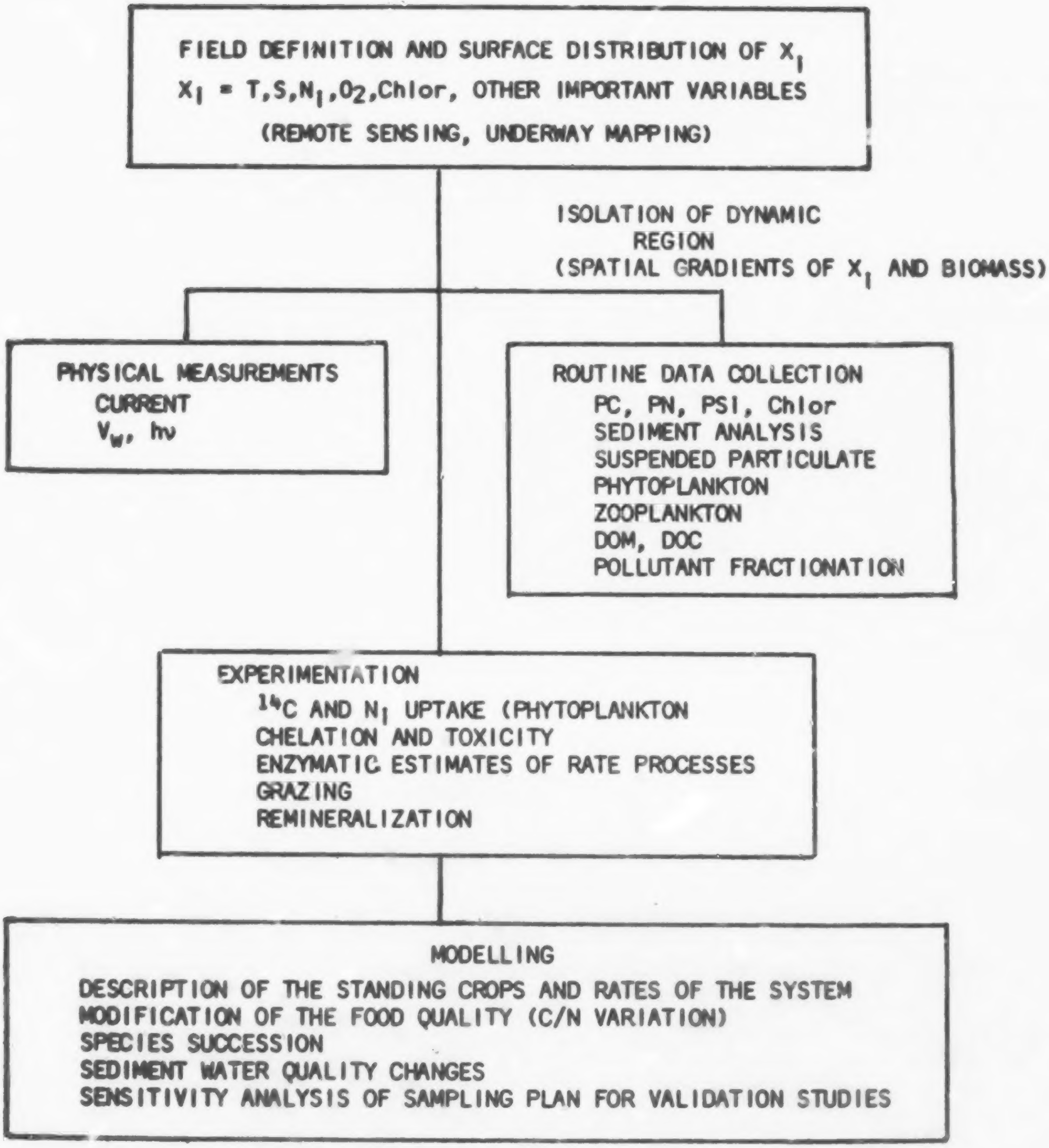

FIELD DEFINITION AND SURFACE DISTRIBUTION OF X_i
X_i = T,S,N_i,O_2,Chlor, OTHER IMPORTANT VARIABLES
(REMOTE SENSING, UNDERWAY MAPPING)
ISOLATION OF DYNAMIC REGION
(SPATIAL GRADIENTS OF X_i AND BIOMASS)
PHYSICAL MEASUREMENTS
CURRENT
V_w, hν
ROUTINE DATA COLLECTION
PC, PN, PSI, Chlor
SEDIMENT ANALYSIS
SUSPENDED PARTICULATE
PHYTOPLANKTON
ZOOPLANKTON
DOM, DOC
POLLUTANT FRACTIONATION
EXPERIMENTATION
^{14}C AND N_i UPTAKE (PHYTOPLANKTON
CHELATION AND TOXICITY
ENZYMATIC ESTIMATES OF RATE PROCESSES
GRAZING
REMINERALIZATION
MODELLING
DESCRIPTION OF THE STANDING CROPS AND RATES OF THE SYSTEM
MODIFICATION OF THE FOOD QUALITY (C/N VARIATION)
SPECIES SUCCESSION
SEDIMENT WATER QUALITY CHANGES
SENSITIVITY ANALYSIS OF SAMPLING PLAN FOR VALIDATION STUDIES

Figure 3. Optimum Analysis Scheme of a Polluted Marine Ecosystem

chlorophyll, etc.) are measured in the area of interest. From this information surface maps of the distributions are produced and hydrographic stations are occupied to define the overall structure in the region. When the most dynamic part of the area has been identified, detailed attention is focused upon it. This phase usually requires 1 to 3 days, depending on the size of the sampling grid.

2) Another continuous record of the surface variables is recorded while the ship steams over a fine grid in the area. Surface contour maps are produced and displayed continuously as the data is acquired and edited (4-10 hours).

3) On the basis of the surface distributions, sites are chosen for experimentation. These sites may be selected because they display certain features such as different limiting nutrient structures, well developed phytoplankton blooms, freshly upwelled water, abrupt gradients of specific pollutants, or for being in the dispersive field of a sewage outfall (4-20 days).

4) When the first set of experiments has begun, the surface distributions are again mapped and new experiments initiated.

5) As soon as the experimental data are available, they are coupled with the distribution data and simulation experiments are initiated. The results of the model prediction are compared with the incoming data and the comparison is evaluated by the scientists in the field.

INTEGRATED SAMPLING AND DATA ACQUISITION/DATA REDUCTION SYSTEM

Interactive Real Time Information System (IRIS)

The various components of the shipboard real time DA/DR system are shown in Figure 4. They include the hull mounted pumping system (HUMPS), the towed underway pumping system (TUPS), salinity-temperature-depth sensors (STD), the hydrographic sampling bottles, radiation and meterological sensors, as well as other auxiliary instrumentation for various data gathering. The AutoAnalyzer and fluorometer receive the samples either continuously or in a batch mode (Figure 2). IRIS collects the information on the peripheral processor through analog to digital conversion and sends it to the central processor for program manipulation and output display. A generalized schematic diagram of IRIS is shown in Figure 5.

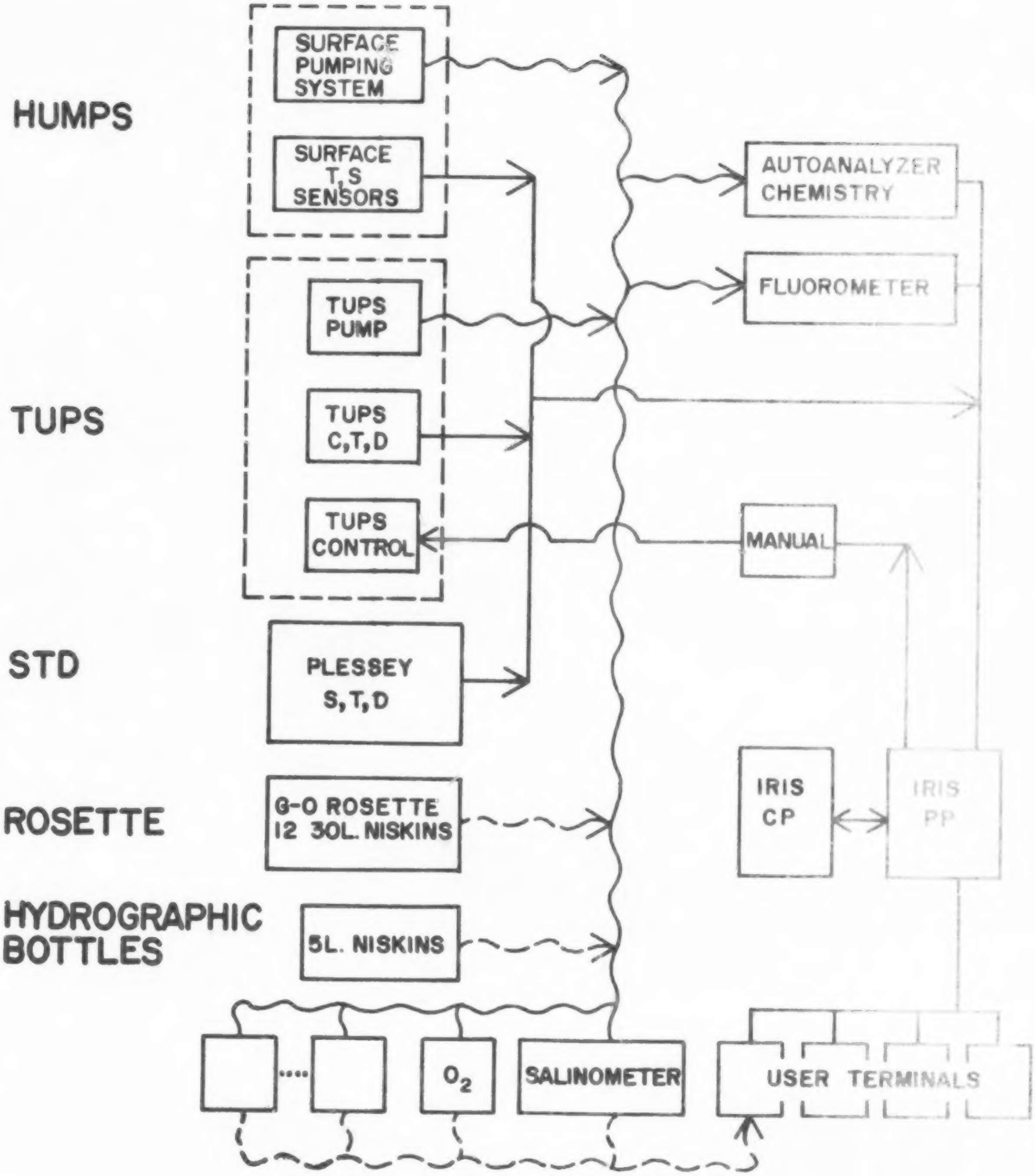

HUMPS
SURFACE PUMPING SYSTEM
SURFACE T,S SENSORS
AUTOANALYZER CHEMISTRY
FLUOROMETER
TUPS
TUPS PUMP
TUPS C,T,D
TUPS CONTROL
MANUAL
STD
PLESSEY S,T,D
ROSETTE
G-O ROSETTE 12 30L. NISKINS
IRIS CP
IRIS PP
HYDROGRAPHIC BOTTLES
5L. NISKINS
O2
SALINOMETER
USER TERMINALS

Figure 4. Schematic Flow Diagram for Biological Sampling

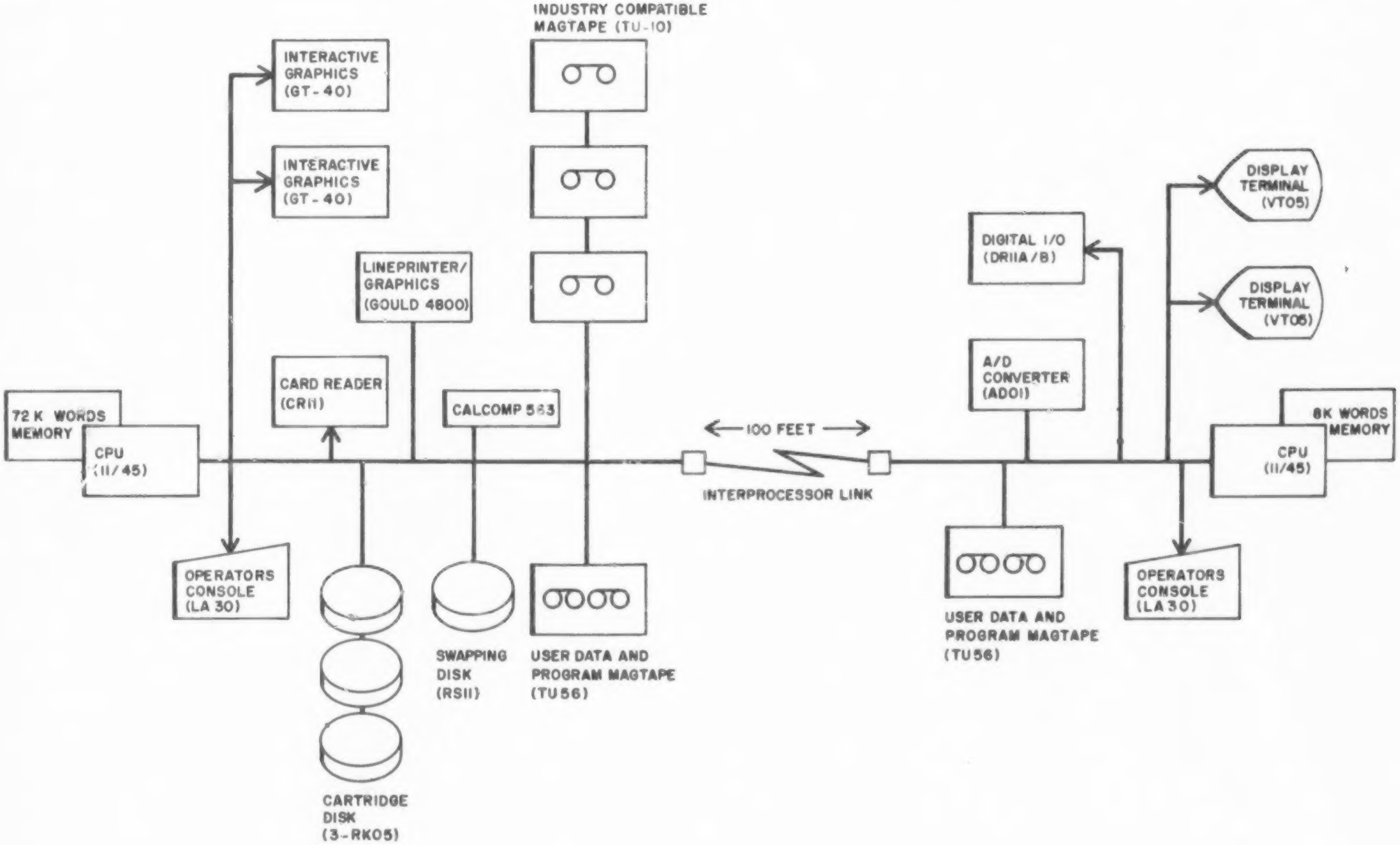

INDUSTRY COMPATIBLE MAGTAPE (TU-10)
INTERACTIVE GRAPHICS (GT-40)
INTERACTIVE GRAPHICS (GT-40)
LINEPRINTER/ GRAPHICS (GOULD 4800)
CARD READER (CR11)
CALCOMP 563
72 K WORDS MEMORY
CPU (11/45)
OPERATORS CONSOLE (LA 30)
CARTRIDGE DISK (3-RK05)
SWAPPING DISK (RS11)
USER DATA AND PROGRAM MAGTAPE (TU56)
← 100 FEET →
INTERPROCESSOR LINK
DIGITAL I/O (DR11A/B)
A/D CONVERTER (AD01)
DISPLAY TERMINAL (VT05)
DISPLAY TERMINAL (VT05)
8K WORDS MEMORY
CPU (11/45)
OPERATORS CONSOLE (LA30)
USER DATA AND PROGRAM MAGTAPE (TU56)

Figure 5. A General Schematic Diagram of the IRIS Computer System

Data stored in the IRIS system is available for output in several forms, depending on the type of data and the user's preference. Together with the standard tabular output, vertical distribution of station data (depth profiles of specific variables), vertical distribution of data from several stations (vertical contour plots), two-dimensional surfaces or subsurface maps of station data (surface contour plots) and two-dimensional underway maps can be constructed. A capability for three-dimensional mapping will also be available in the future. All of these forms of output may be displayed on an oscilloscope console or obtained as hard copy on a printer. Examples of typical data output are shown in Figures 15 and 26, with a discussion of the observations following in the Shipboard Experimentation section.

Towed Underway Pumping System (TUPS)

The "traditional" sampling procedure, as shown in Figure 6, involves continuous underway surface mapping to determine the horizontal distribution of temperature, salinity, nutrient and flourescence. This operation is conducted at night. By compiling this information with the sampling data obtained during the day, the vertical distribution of these properties is defined. To alleviate this time consuming process, we have designed a towed underway pumping system (TUPS) which has a three-dimensional mapping capability. A typical sampling cycle with TUPS is shown in Figure 7.

The ship steams the normal zig-zag cruise track while an active depressor (in our case, a model 3000 Batfish, Hermes Electronics, Ltd.), oscillates vertically in a sinusoidal fashion from the surface to a maximum depth of 100 meters. The depressor was chosen to provide a variable depth capability without having excessive cable wear and to minimize the strength and weight requirements of the storage and retrieval reel. The Batfish contains a hydraulic servo-control mechanism, a Guildline CTD and a Deming water pump. The system can be towed at 10 knots over a depth range of 0-110 meters while pumping water up to deck at 10 gal/min under a pressure of 10 lbs/in . The pumping hoses are made of polyurethane and are free from synthetic organics or trace metal contaminants. The CTD in the Batfish is accurate to ± .04 0/00 in salinity units and ± .02°C.

The whole system consists of a winch (used only for storage, deployment and retrieval), a faired cable containing the pumping hoses, electrical conductors and a coaxial cable (Figure 8), the Batfish, and the emergency recovery system. The Batfish is towed with an approximate 3

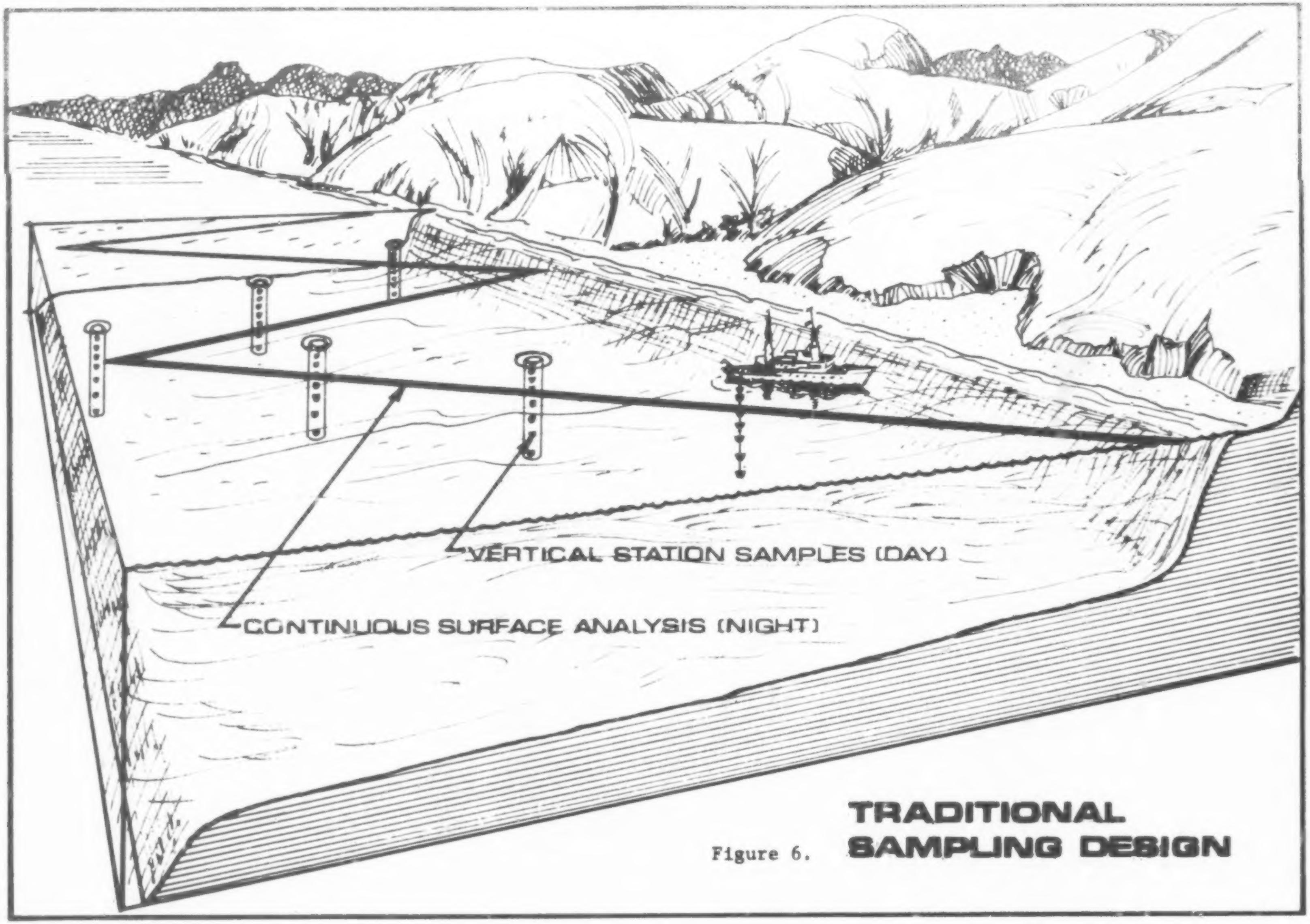
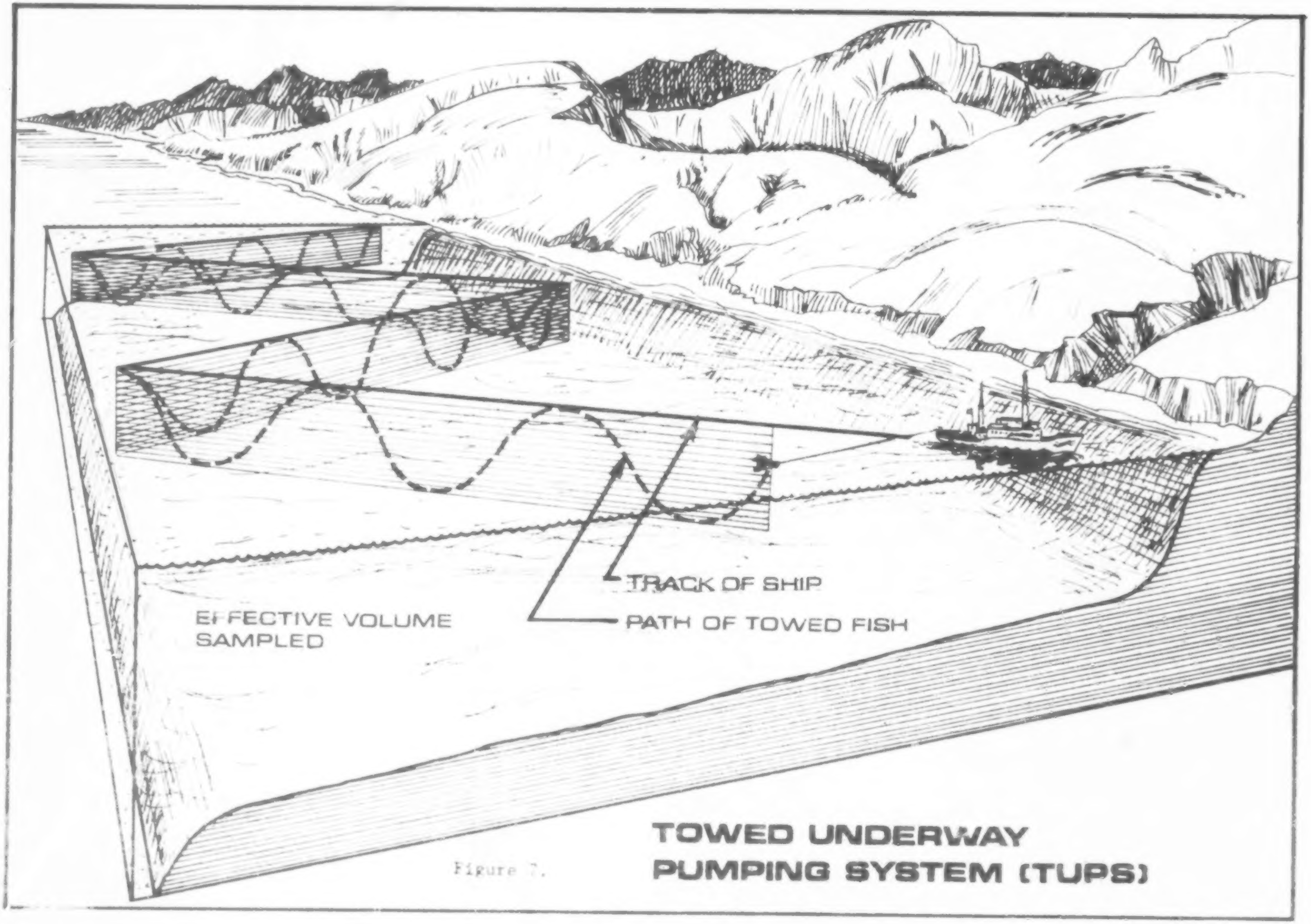

VERTICAL STATION SAMPLES (DAY)
CONTINUOUS SURFACE ANALYSIS (NIGHT)

Figure 6. **TRADITIONAL SAMPLING DESIGN**


EFFECTIVE VOLUME SAMPLED
TRACK OF SHIP
PATH OF TOWED FISH

TOWED UNDERWAY PUMPING SYSTEM (TUPS)

Figure 7.

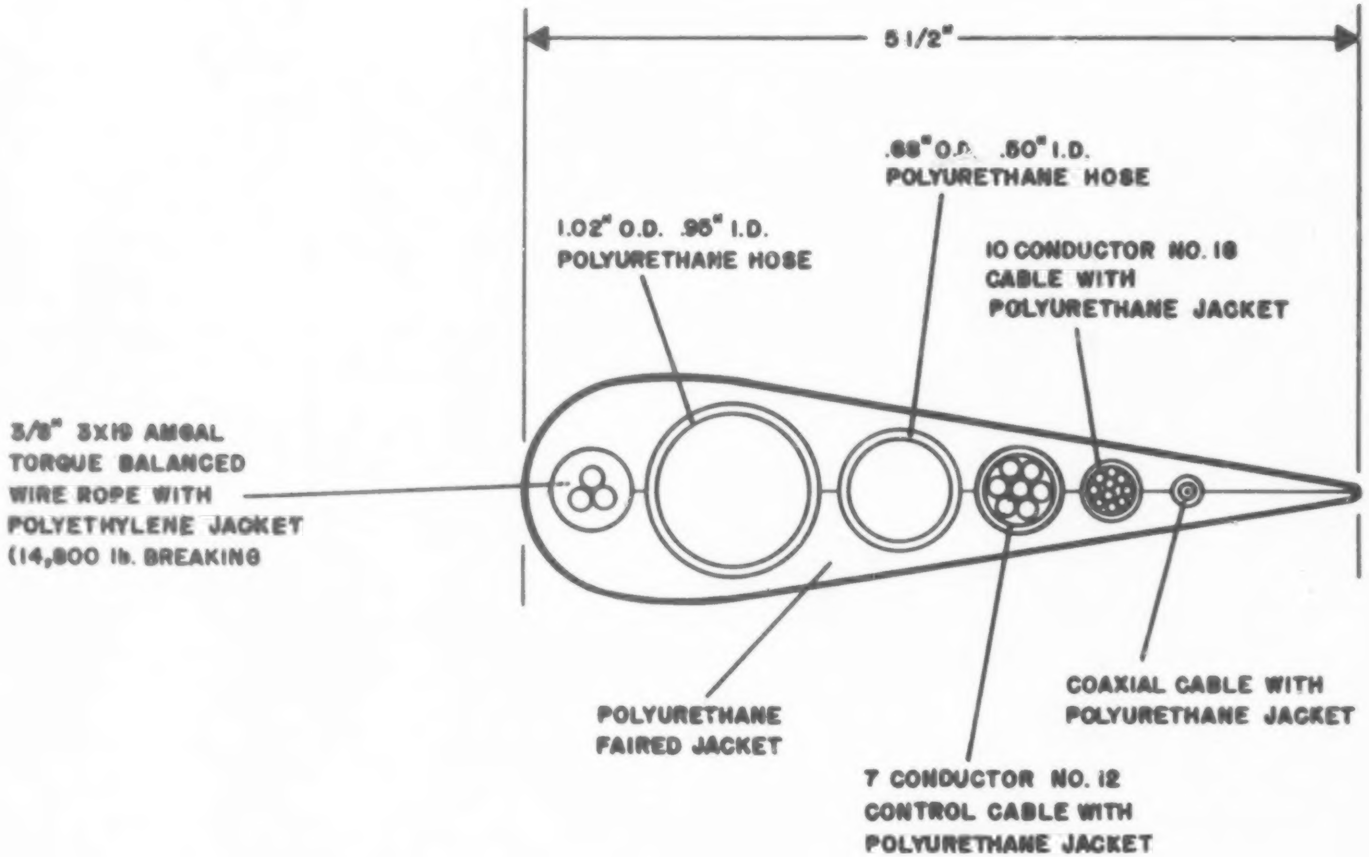

5 1/2"
1.02" O.D. .95" I.D.
POLYURETHANE HOSE
.68" O.D. .50" I.D.
POLYURETHANE HOSE
10 CONDUCTOR NO. 18
CABLE WITH
POLYURETHANE JACKET
3/8" 3X19 AMGAL
TORQUE BALANCED
WIRE ROPE WITH
POLYETHYLENE JACKET
(14,800 lb. BREAKING
POLYURETHANE
FAIRED JACKET
COAXIAL CABLE WITH
POLYURETHANE JACKET
7 CONDUCTOR NO. 12
CONTROL CABLE WITH
POLYURETHANE JACKET

Figure 8. Specifications of the Tups Faired Cable

to 1 scope, as shown in Figure 9. The overall deck configuration of TUPS is shown in Figure 10 and a block diagram of the power control components is displayed in Figure 11.

PHYSICAL MEASUREMENTS

The physical description of a polluted marine region and the definition of the physical processes governing the distribution of discharged material are important factors in establishing a successful monitoring program.

Data on the circulation patterns of the study area are necessary for obtaining information on the residence times of discharged microconstitutents, describing the flushing characteristics of the area, and correlating pollutant accumulation with the physical state of the system.

We usually obtain information on the circulation features of the area by deploying drogues for monitoring the water movements. The typical drogue consists of a parachute or cross (acting as a sea anchor) attached to a float surrounding a metal pole manifold equipped with a light and radar reflector. The ship follows the drogue and positions are noted every 1 or 2 hours. After a few tidal cycles, the gross circulation pattern can be deduced. Anchored current meters are also deployed occasionally to measure speed and direction of currents at a fixed location. Progressive vector diagrams from the current meter data are then constructed for interpretation.

CHEMICAL AND BIOLOGICAL DATA COLLECTION

Numerous samples for both chemical and biological analyses are collected routinely at the sampling stations. Samples collected from 5-liter water sampling bottles are used for salinity, oxygen, nitrate, nitrite, ammonium, silicate, phosphate, and trace metal analyses. Temperature data are obtained with reversing thermometers. Phytoplankton samples collected with twelve 30-liter water samplers, in a rosette configuration, are used for phytoplankton standing stock data and isotopic tracer experiments. Biomass is determined by routinely measuring chlorophyll, particulate carbon, particulate nitrogen, particulate silica, respiratory electron transport activity (ETS) and total amount of particulate matter in the form of a size-frequency specturm. Zooplankton and sediments are also collected for trace organic analysis. Sampling methodology for plankton includes standard net techniques as well as special devices such as the Large Volume Filter

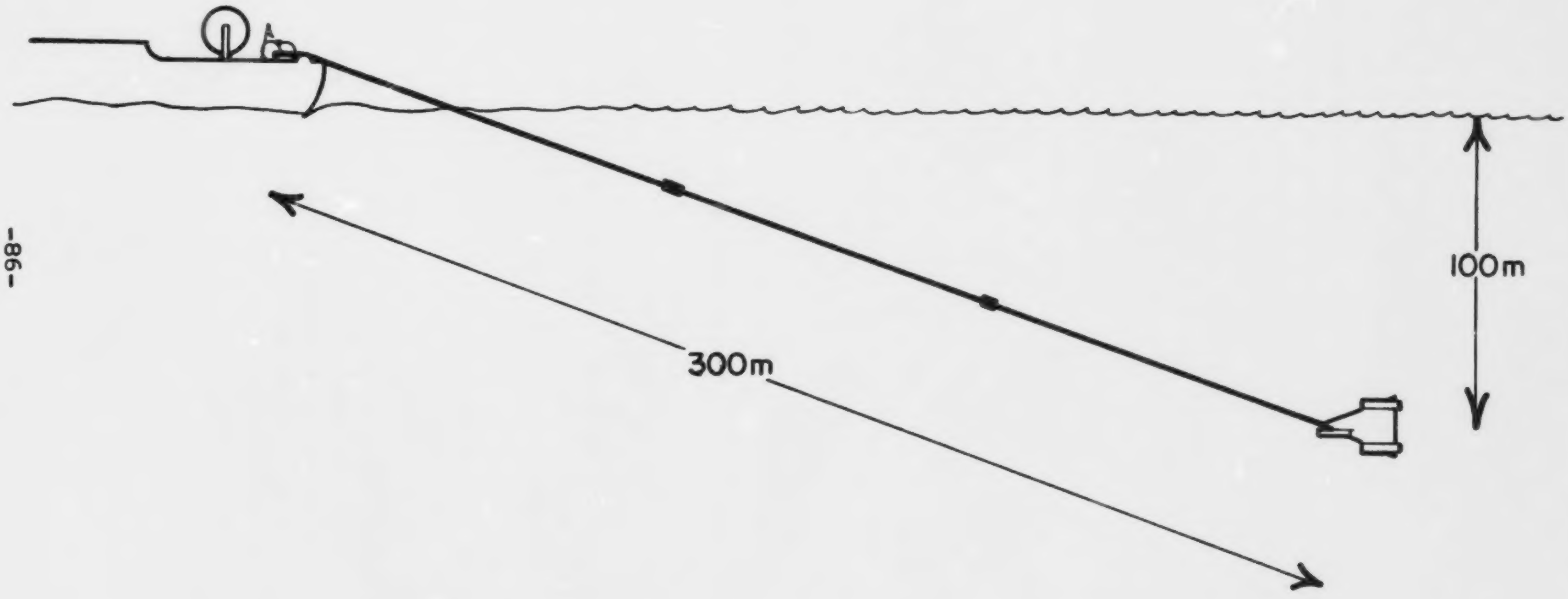


Figure 9. Towing Configuration of the Batfish

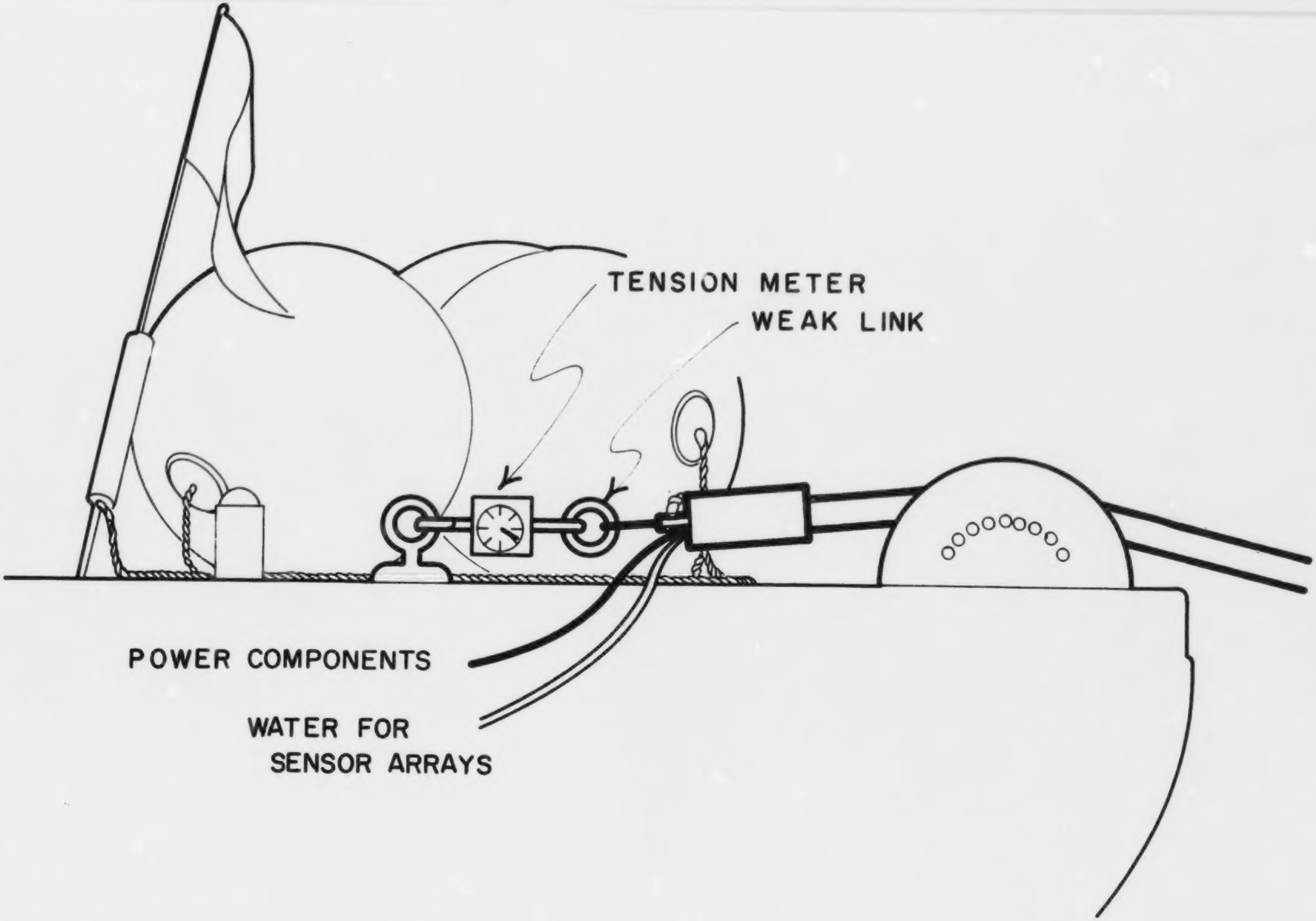

TENSION METER
WEAK LINK
POWER COMPONENTS
WATER FOR SENSOR ARRAYS

Figure 10. The Overall Deck Configuration of Tups

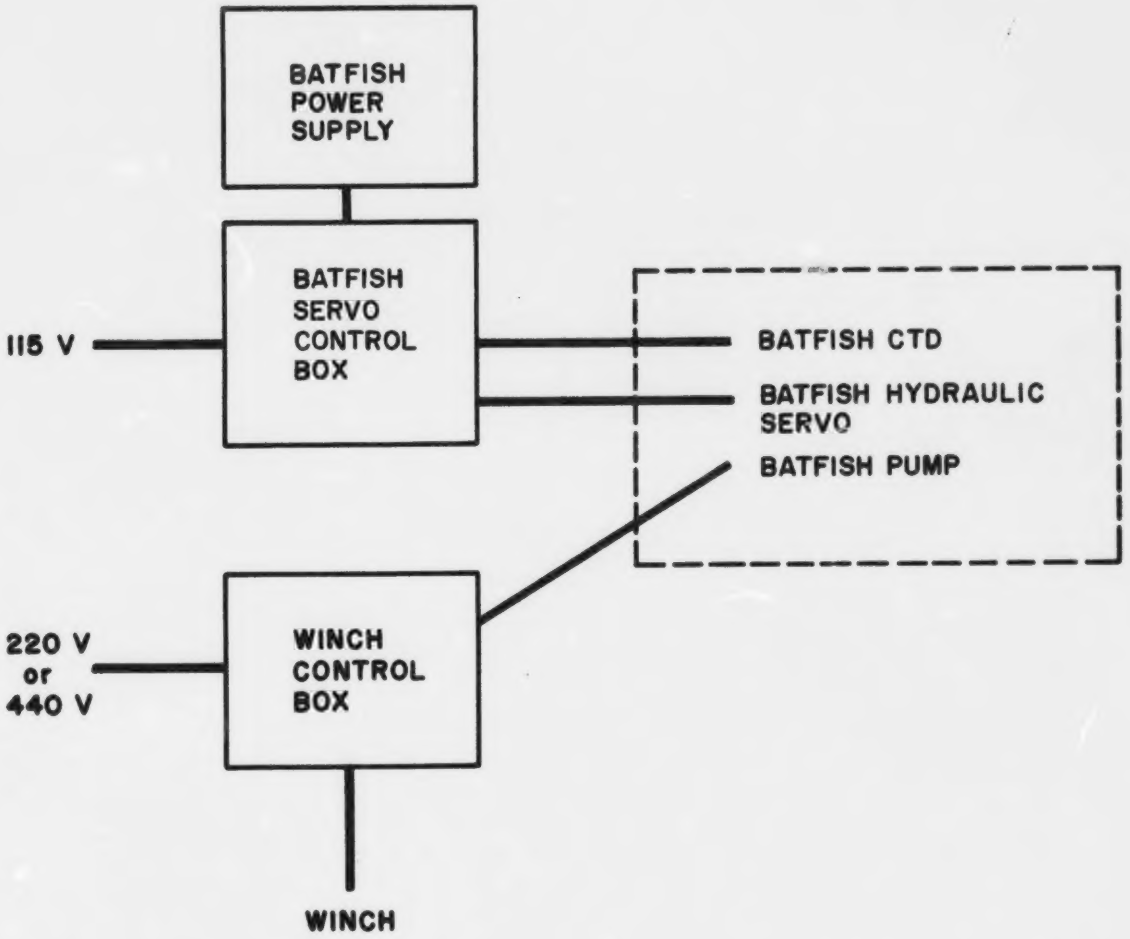

BATFISH POWER SUPPLY
BATFISH SERVO CONTROL BOX
115 V
BATFISH CTD
BATFISH HYDRAULIC SERVO
BATFISH PUMP
220 V or 440 V
WINCH CONTROL BOX
WINCH

Figure 11. Block Diagram of the TUPS Power Control Components

(LVF) and the Protected Closed Net (PCN) for phytoplankton and zooplankton collections respectively, as used in our trace organics program. Schematic diagrams of these devices are shown in Figures 12 and 13.

SHIPBOARD EXPERIMENTATION

Assessment of the biological impact of marine outfalls on the primary productivity of the area has been accomplished by following photosynthetic and respiratory activity as well as changes in phytoplankton nutrient uptake. Radioactive tracers and stable isotopes have been commonly used to monitor these rate processes. Shipboard toxicity studies include growing of natural phytoplankton populations in the ambient water and monitoring changes in ^{14}C, ^{15}N-uptake and respiration rates; observing the approach of laboratory cultured phytoplankton and zooplankton to toxicity threshold behavior under the actual trace compound load of the system; pulsing of natural populations with higher trace compound concentration in reference to the ambient levels and monitoring their growth response and their adaptability to the perturbation.

Carbon fixation is measured by the traditional ^{14}C method with shipboard analysis carried out by a gas flow geiger counter or a scintillation counter. Nitrogen and silica utilization is determined by nutrient uptake experiments with ^{15}N and ^{29}Si tracers. The isotopes are analyzed on a shipboard mass spectrometer. Incubation experiments are performed onboard ship using standard procedures associated with the simulated *in situ* methods.

Trace organic (chlorinated hydrocarbons) accumulation in phytoplankton, suspended particulate matter, zooplankton and sediments are measured by shipboard gas chromatography and confirmed by mass spectrometry. Anodic stripping voltametry has been used occasionally onboard ship for some trace metal determination in seawater (e.g., Zn, Cu, Pb).

SOME PRELIMINARY RESULTS

The data presented below can be used as an example of the inherent advantages in adopting a systems and real time approach to marine pollution monitoring. The area investigated is the coastal waters of the Southern California Bight, off the White's Point Outfall in Los Angeles (OUTFALL I, II cruise series, 1972-73, R/V T.G. Thompson). A general map of the region, together with station locations during the OUTFALL-II studies is shown in Figure 14.

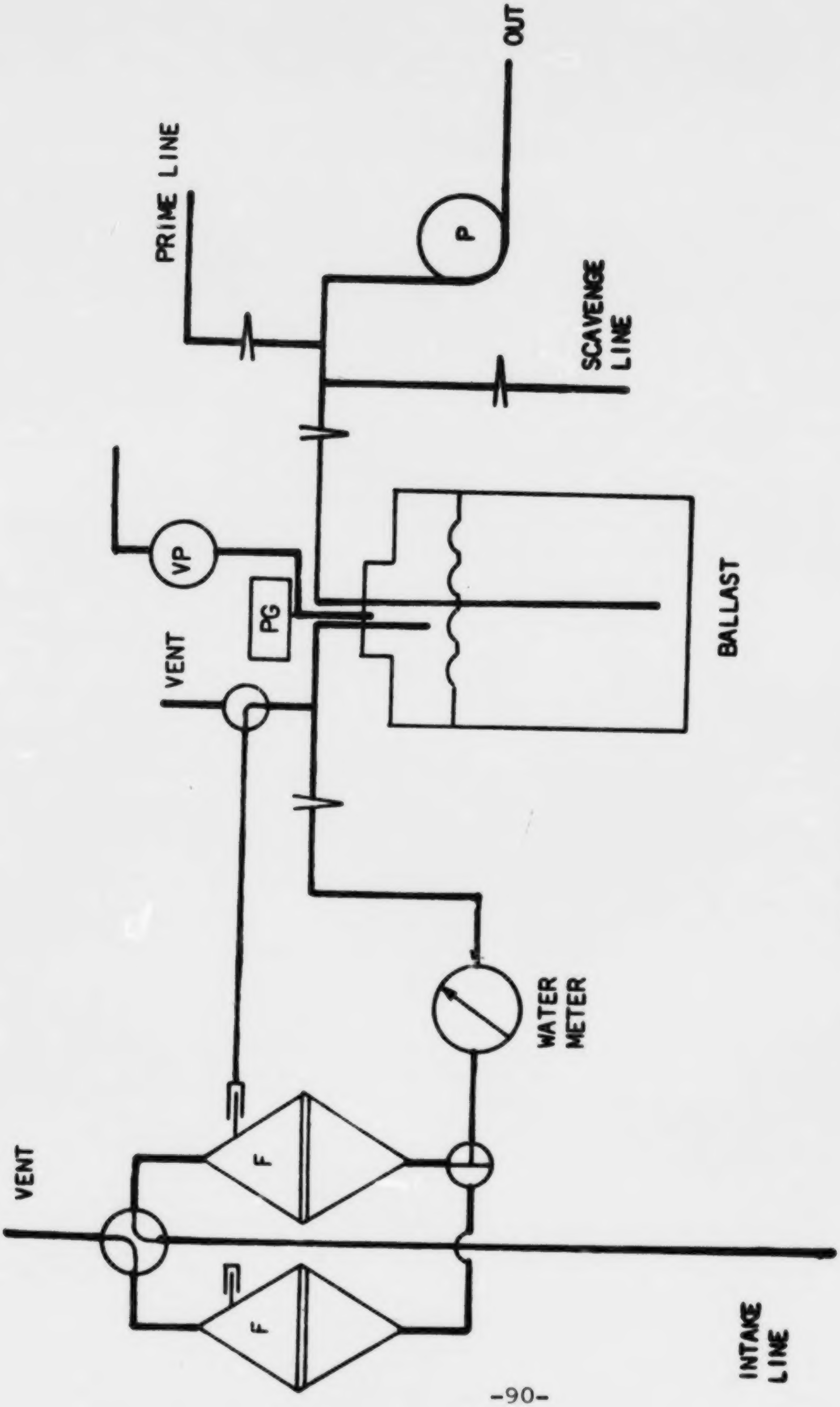

PRIME LINE
OUT
P
SCAVENGE LINE
VP
PG
VENT
BALLAST
WATER METER
VENT
F
F
INTAKE LINE

Figure 12. **SCHEMATIC DIAGRAM OF THE HV FILTER**

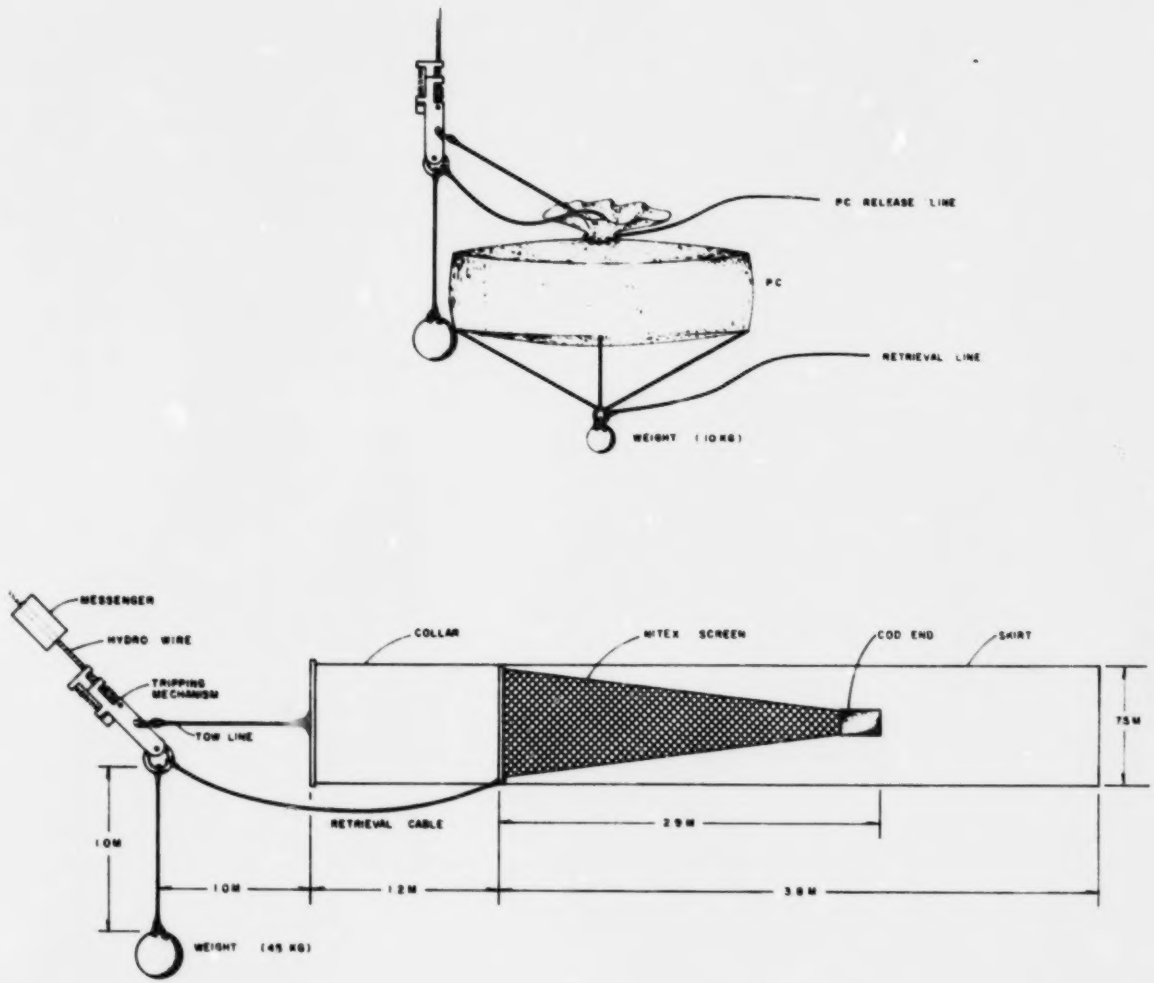

PC RELEASE LINE
PC
RETRIEVAL LINE
WEIGHT (10KG)
MESSENGER
HYDRO WIRE
TRIPPING MECHANISM
TOW LINE
COLLAR
NITEX SCREEN
COD END
SKIRT
75M
RETRIEVAL CABLE
29M
10M
10M
12M
38M
WEIGHT (45 KG)

Figure 13. Configuration of the PCN
(a) Landing stage
(b) Towlng stage

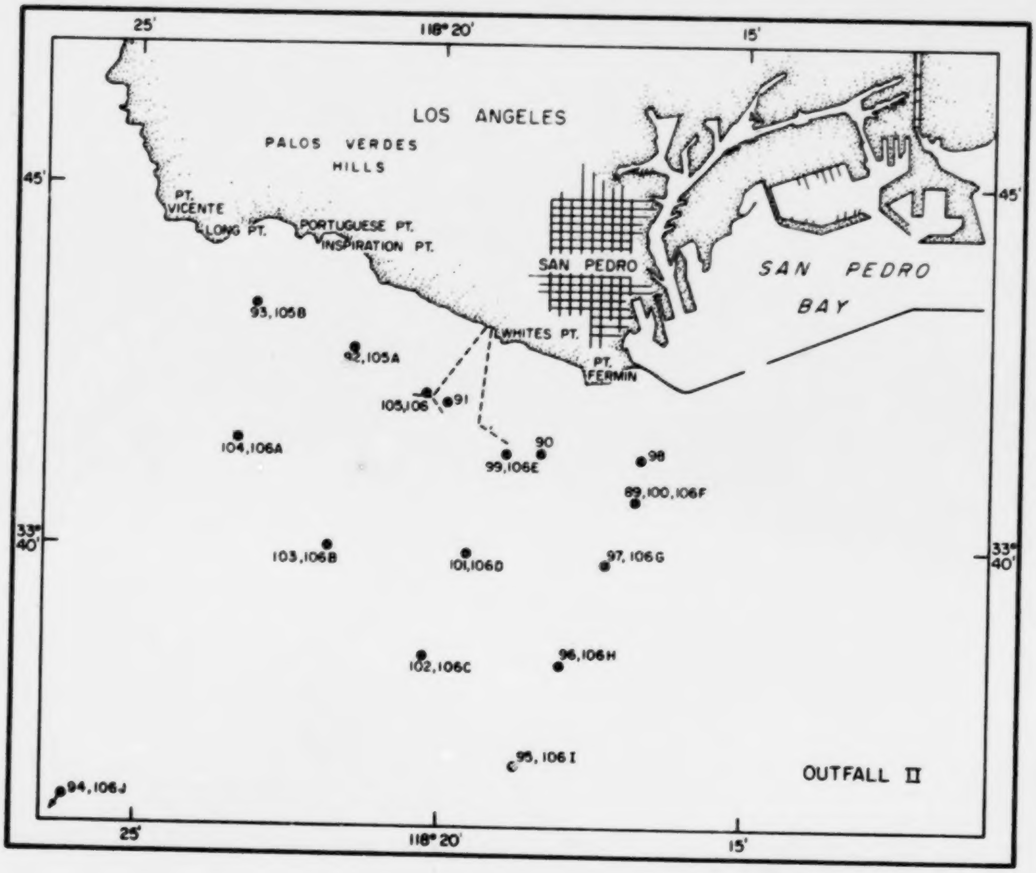

25'
118° 20'
15'
LOS ANGELES
PALOS VERDES HILLS
45'
PT. VICENTE
LONG PT.
PORTUGUESE PT.
INSPIRATION PT.
SAN PEDRO
SAN PEDRO BAY
WHITES PT.
PT. FERMIN
93, 105B
92, 105A
105, 106
91
104, 106A
90
99, 106E
98
89, 100, 106F
33° 40'
103, 106B
101, 106D
97, 106G
102, 106C
96, 106H
95, 106I
94, 106J
OUTFALL II

Figure 14. Station Locations During OUTFALL-11 Cruise

The major objectives of these investigations were to evaluate the extent of the submerged sewage field, to assess the degree to which the dispersive field might influence the biological productivity of the area, and to determine whether the sewage field is affected by upwelling.

Following a brief aerial survey (2-4 hrs) for locating distinct surface water and biomass structures in terms of temperature and chlorophyll gradients, a preliminary field analysis was carried out with all underway surfaces sensors in operation.

Typical grid maps for temperature, nutrients and chlorophyll fluorescence are shown in Figures 15 through 21. Although temperature, phosphate, nitrate, nitrite and siliate show very little variation in the vicinity of the outfall, chlorophyll fluorescence and ammonium concentration display distinct maxima. These surface maps are produced immediately after completing the grid so that the output data may be used in planning the location of hydrographic and productivity stations in the most dynamic region of the area.

The submerged sewage field was located by utilizing salinity-temperature-depth (STD) sensor array data similar to the profile shown in Figure 22. The observed temperature and salinity values were used to calculate the density, denoted as where = (density-1) x 1000 on the plot. Station 093, located near Long Point, shows a characteristic profile of low salinity water at about 7 meters, corresponding to the thermocline depth and reflecting the position of the submerged field. Other STD station data also confirm the general presence of the sewage field in the thermocline region.

Vertical contour plots of nutrients for a longshore transect are shown in Figures 23 to 26. The distribution characteristics correlate well with the STD data. However, nitrate and silicate show reduced sensitivity in locating the sewage field when compared to ammonium or phosphate. Ammonium is a good tracer for the discharge effluent because of low natural background and high concentrations in the discharged effluent.

The concentration of ammonium is so large in the sewage field that it may still be apparent after other discharged substances have been reduced to background values. Ammonium concentrations of five times the normal, or twice the maximum oceanic values have been observed in areas where no

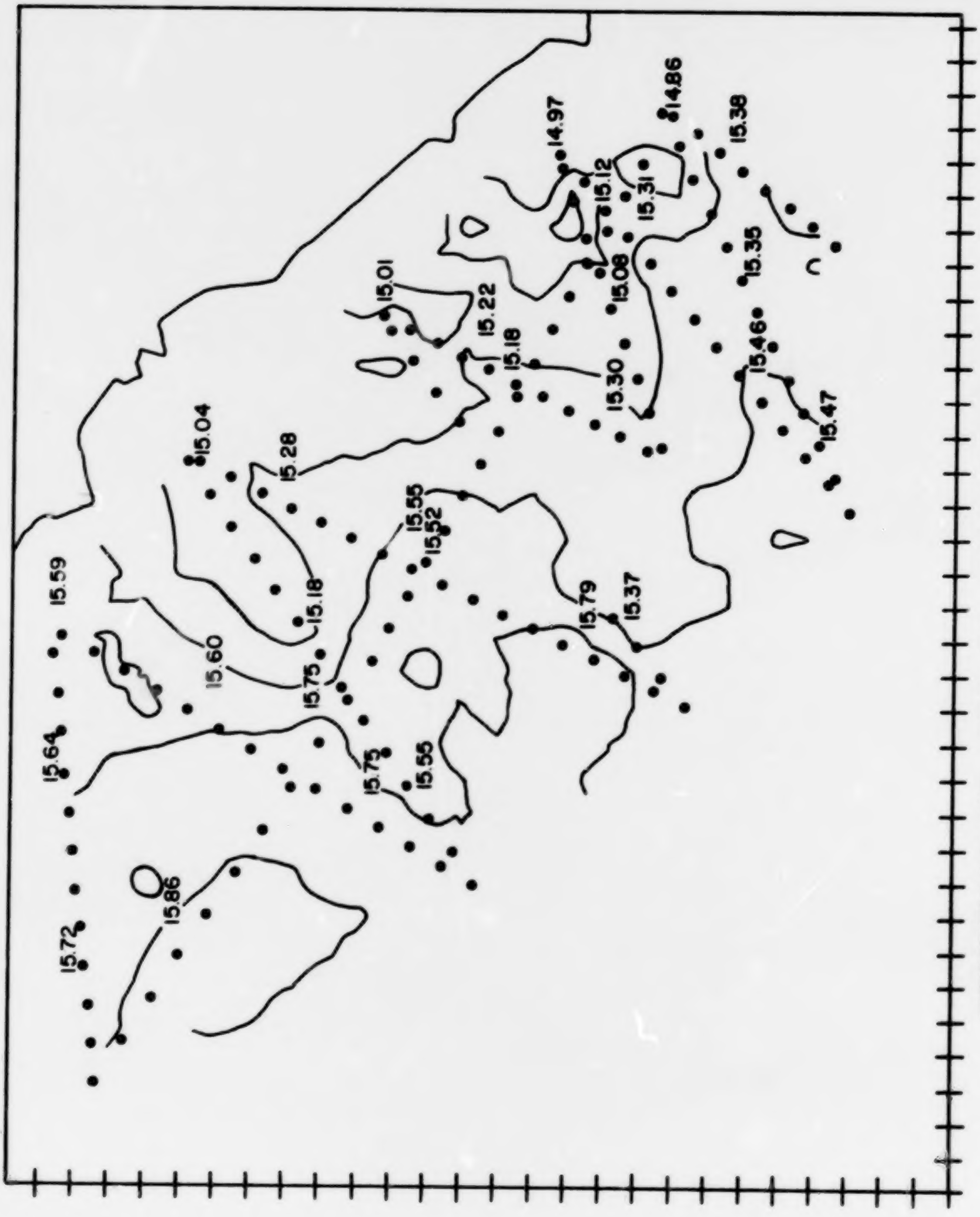

15.59
15.64
15.72
15.86
15.60
15.75
15.18
15.04
15.28
15.55
15.52
15.75
15.55
15.79
15.37
15.01
15.22
15.18
15.30
14.97
15.12
15.08
15.31
14.86
15.38
15.35
15.46
15.47

Figure 15. TEMPERATURE - GRID 28, 7 MAY 73. R=5
CONTOUR INTERVAL = 0.200

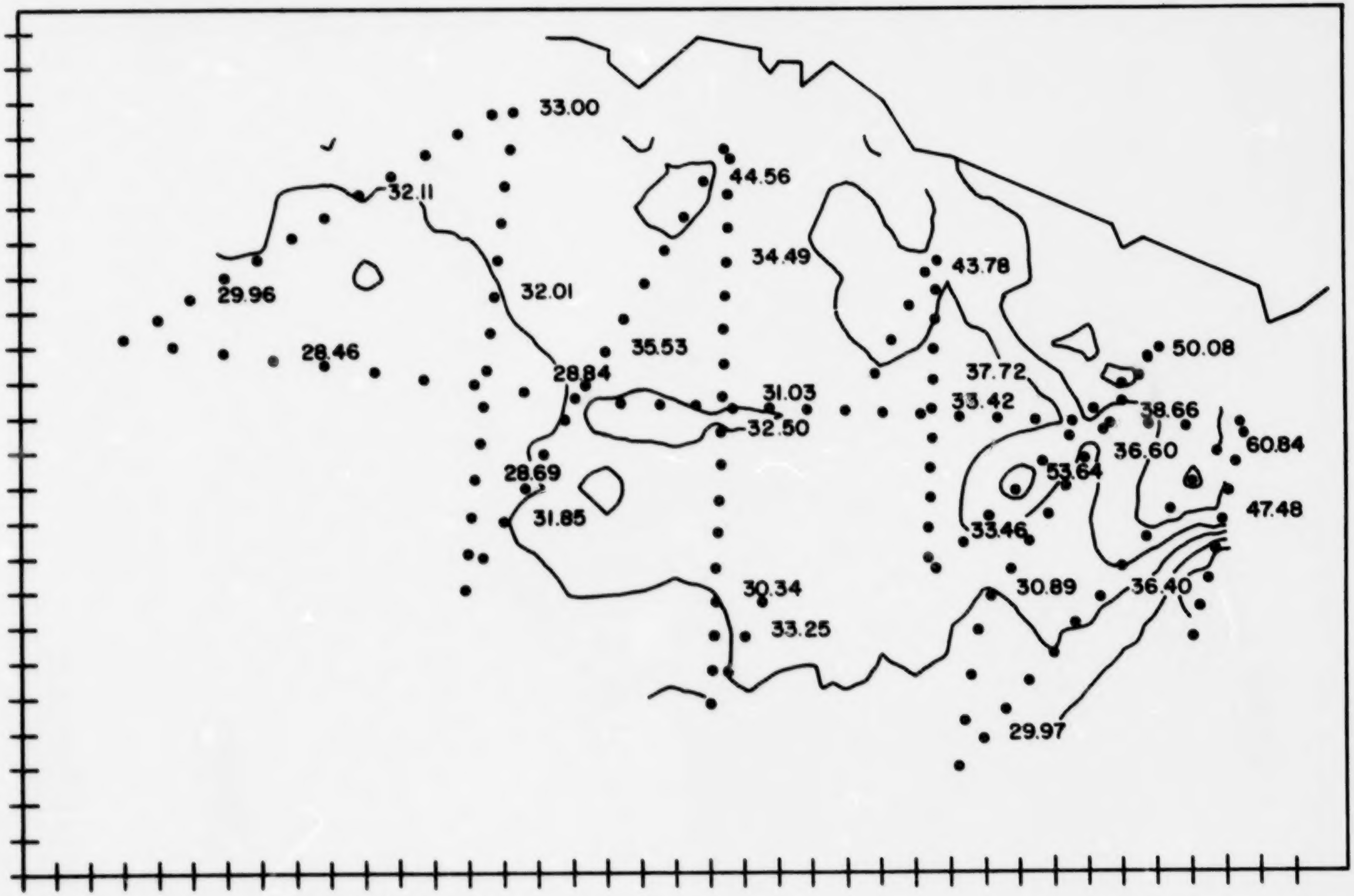

33.00
32.11
44.56
34.49
43.78
29.96
32.01
28.46
35.53
50.08
37.72
28.84
31.03
33.42
38.66
32.50
36.60
60.84
53.64
28.69
31.85
33.46
47.48
30.34
30.89
36.40
33.25
29.97

Figure 16. CHL - GRID 28, 7 MAY 73. R=5
CONTOUR INTERVAL = 8.000

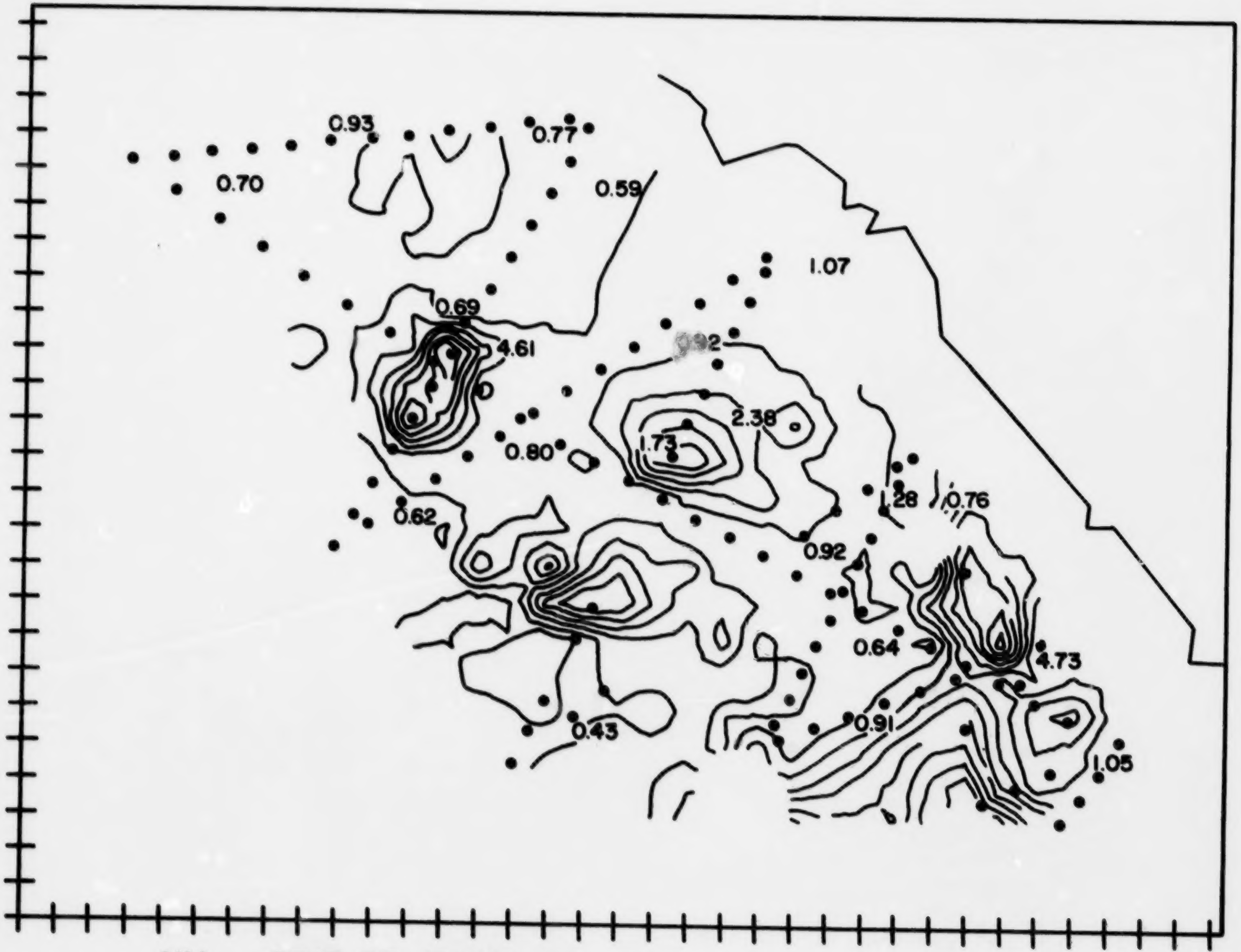

0.93
0.77
0.70
0.59
1.07
0.69
4.61
0.92
2.38
1.73
0.80
1.28
0.76
0.62
0.92
0.64
4.73
0.91
0.43
1.05

Figure 17. NH_4 - GRID 28, 7 MAY 73. R=5

CONTOUR INTERVAL = 0.400

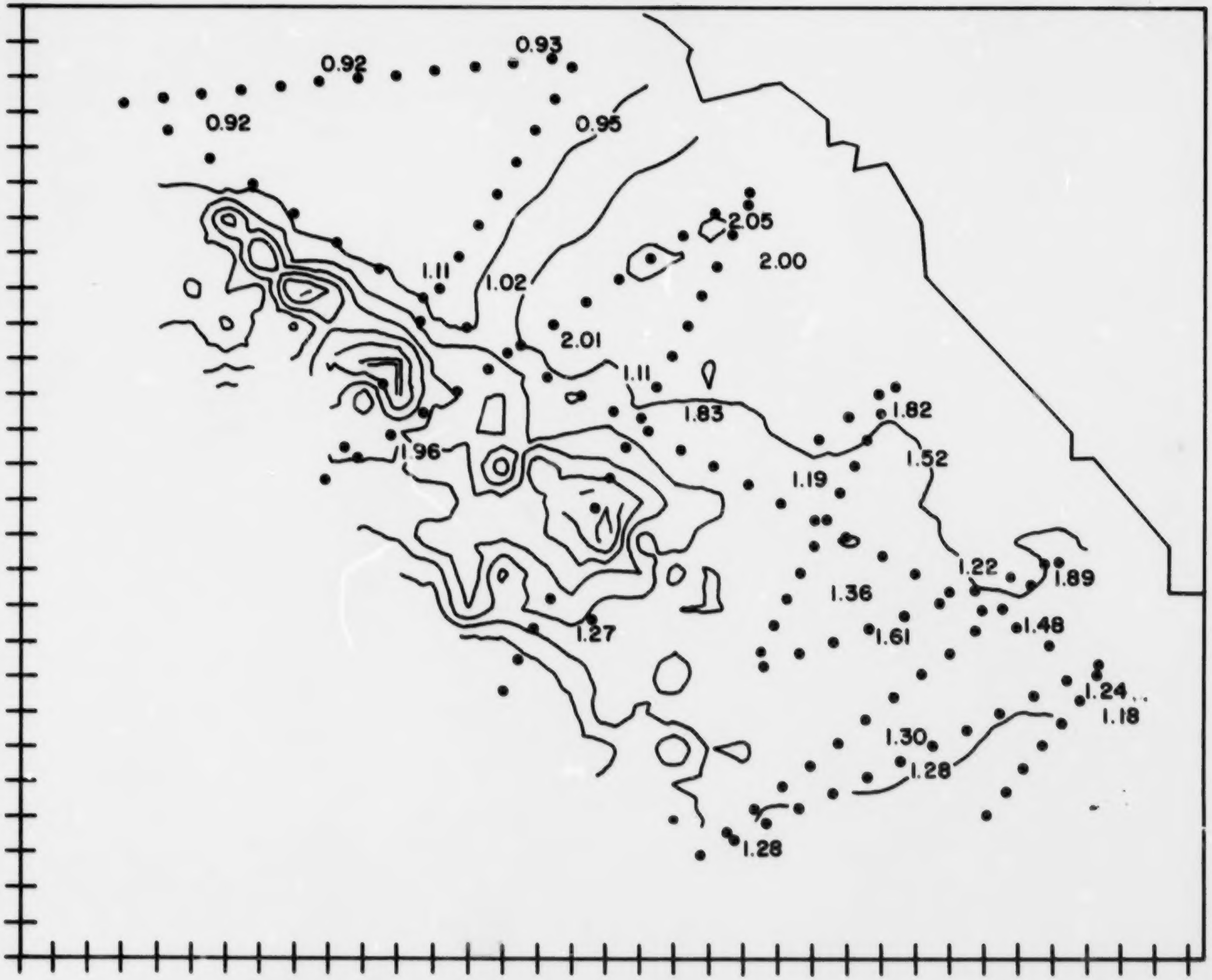

0.93
0.92
0.92
0.95
1.11
1.02
2.05
2.00
2.01
1.11
1.83
1.82
1.52
1.96
1.19
1.22
1.89
1.36
1.48
1.61
1.27
1.24
1.18
1.30
1.28
1.28

Figure 18. PO$_4$ - GRID 28, 7 MAY 73. R=5
CONTOUR INTERVAL = 0.400

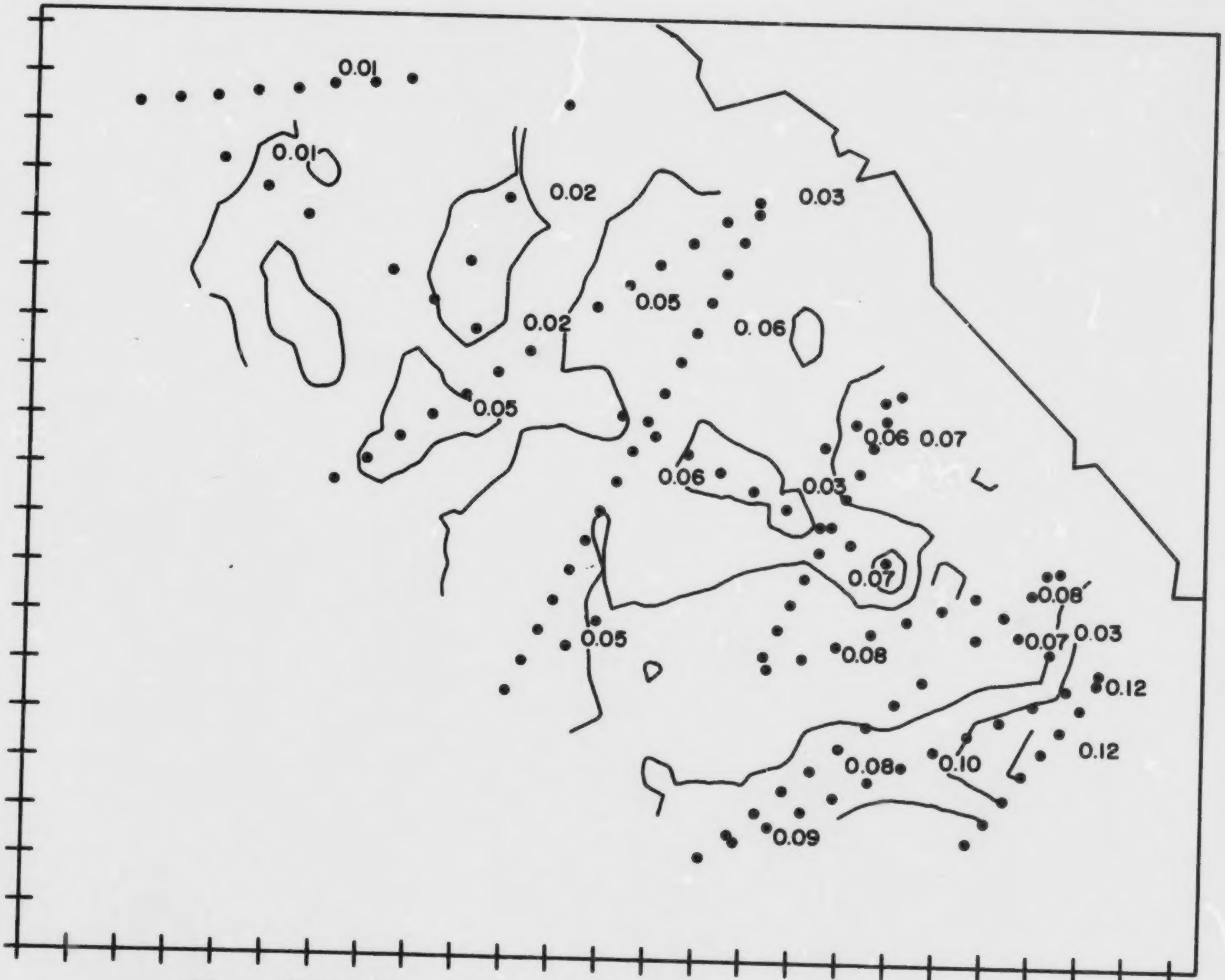

0.01
0.01
0.02
0.02
0.03
0.05
0.06
0.05
0.06
0.06
0.07
0.03
0.07
0.08
0.07
0.03
0.05
0.08
0.12
0.08
0.10
0.12
0.09

Figure 19. NO_3 - GRID 28, 7 MAY 73. R=5
CONTOUR INTERVAL = 0.020

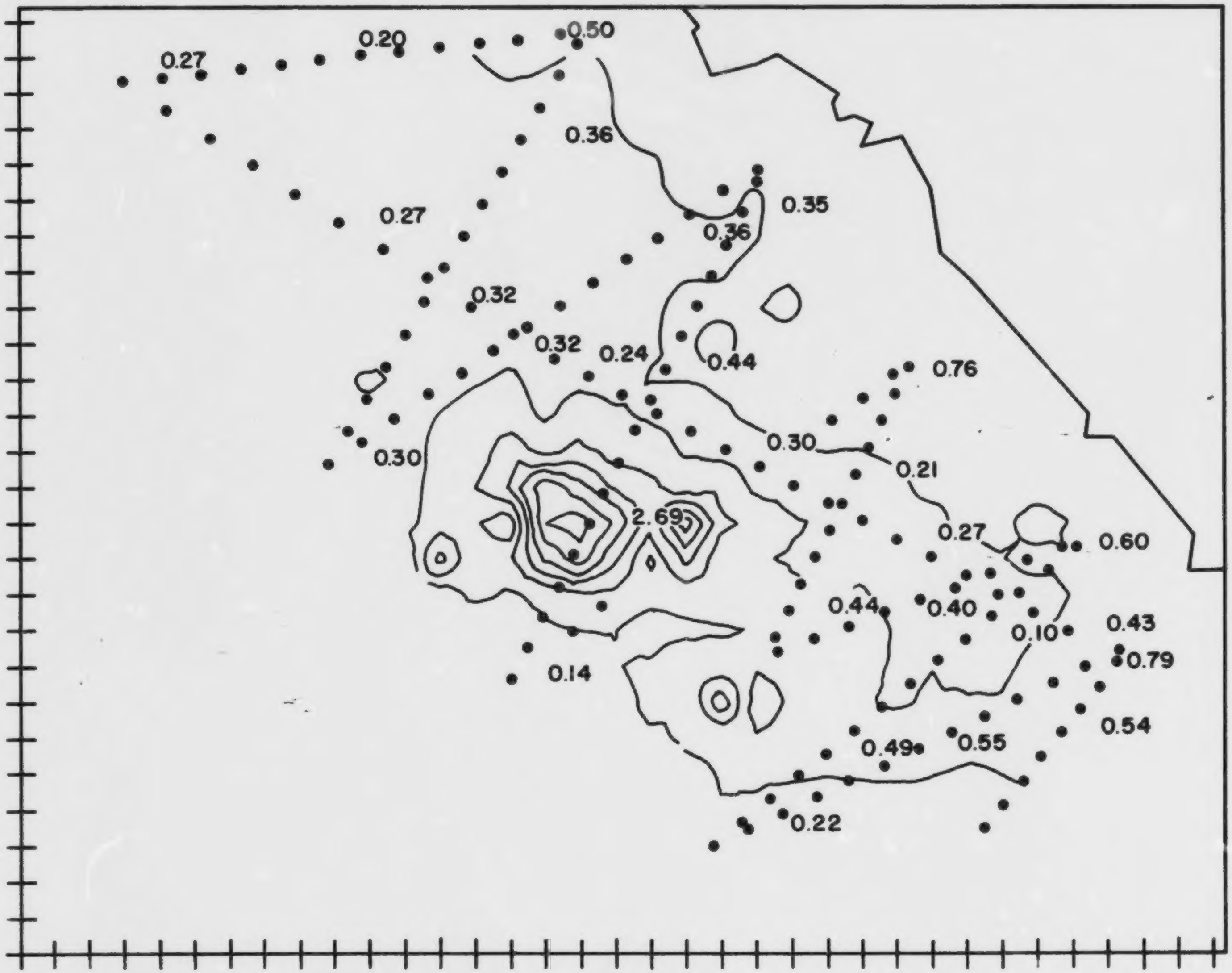

0.27
0.20
0.50
0.36
0.35
0.36
0.27
0.32
0.32
0.24
0.44
0.76
0.30
0.30
0.21
2.69
0.27
0.60
0.44
0.40
0.10
0.43
0.79
0.14
0.49
0.55
0.54
0.22

Figure 20. NO_2 -GRID 28, 7 MAY 73. R=5
CONTOUR INTERVAL = 0.400

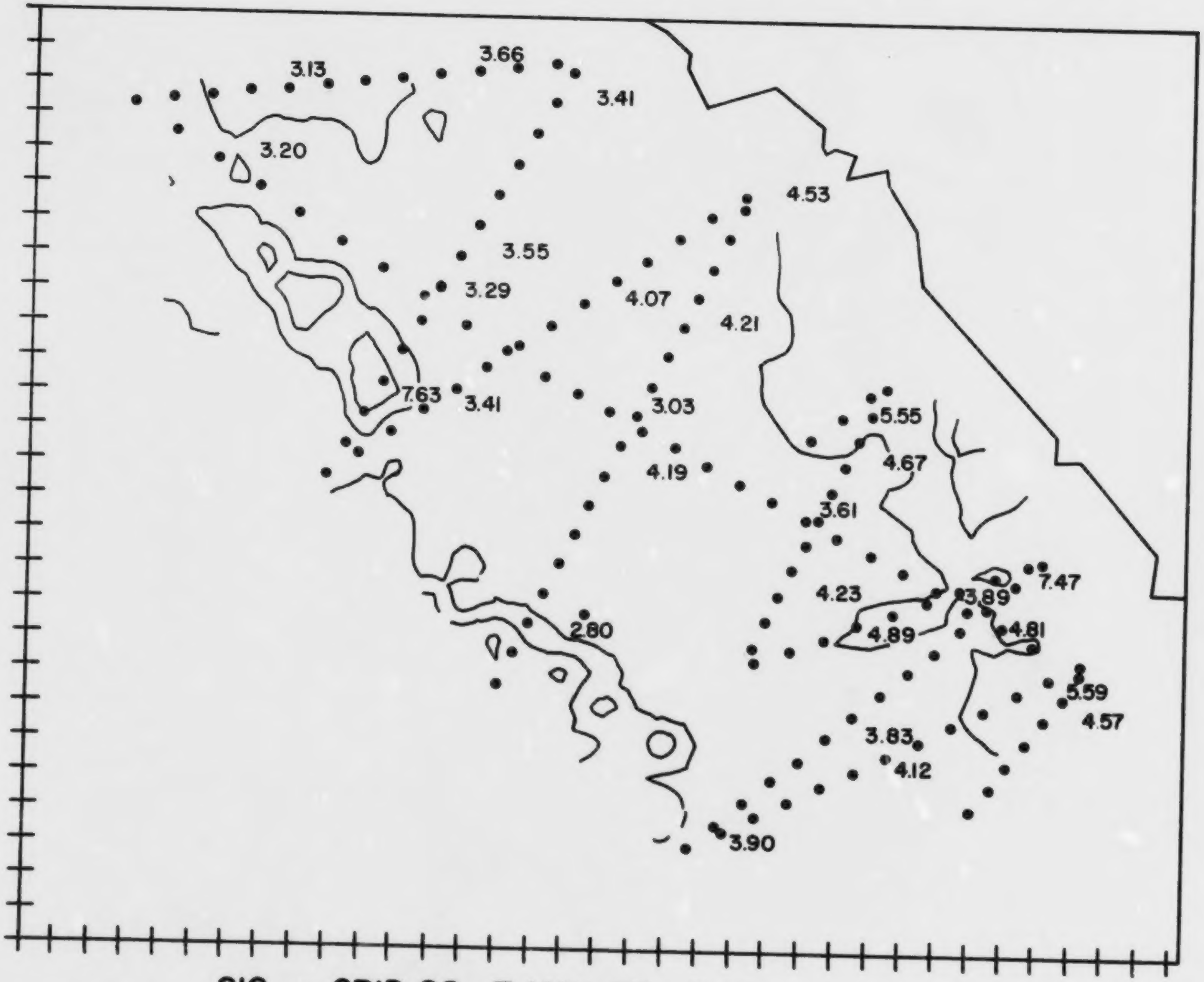

3.13
3.66
3.41
3.20
3.55
3.29
4.53
4.07
4.21
7.63
3.41
3.03
5.55
4.19
4.67
3.61
4.23
7.47
3.89
4.81
2.80
4.89
5.59
4.57
3.83
4.12
3.90

Figure 21. SIO_4 - GRID 28, 7 MAY 73. R = 5
CONTOUR INTERVAL = 1.600

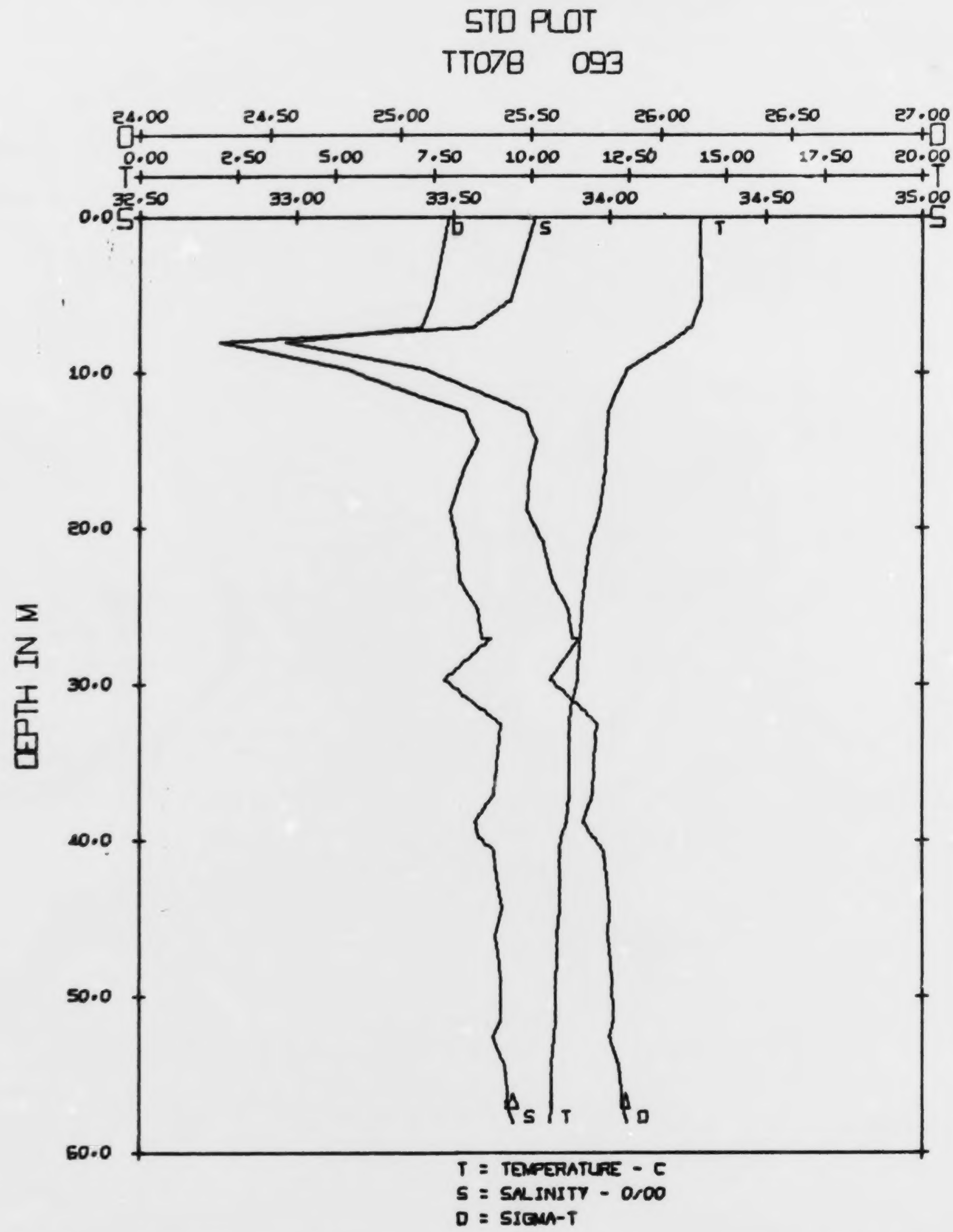


FIGURE 22. LATITUDE 33-43.4
LONGITUDE 118-23.0

DATE 8 MAY 1973
BEGIN 500 END 500

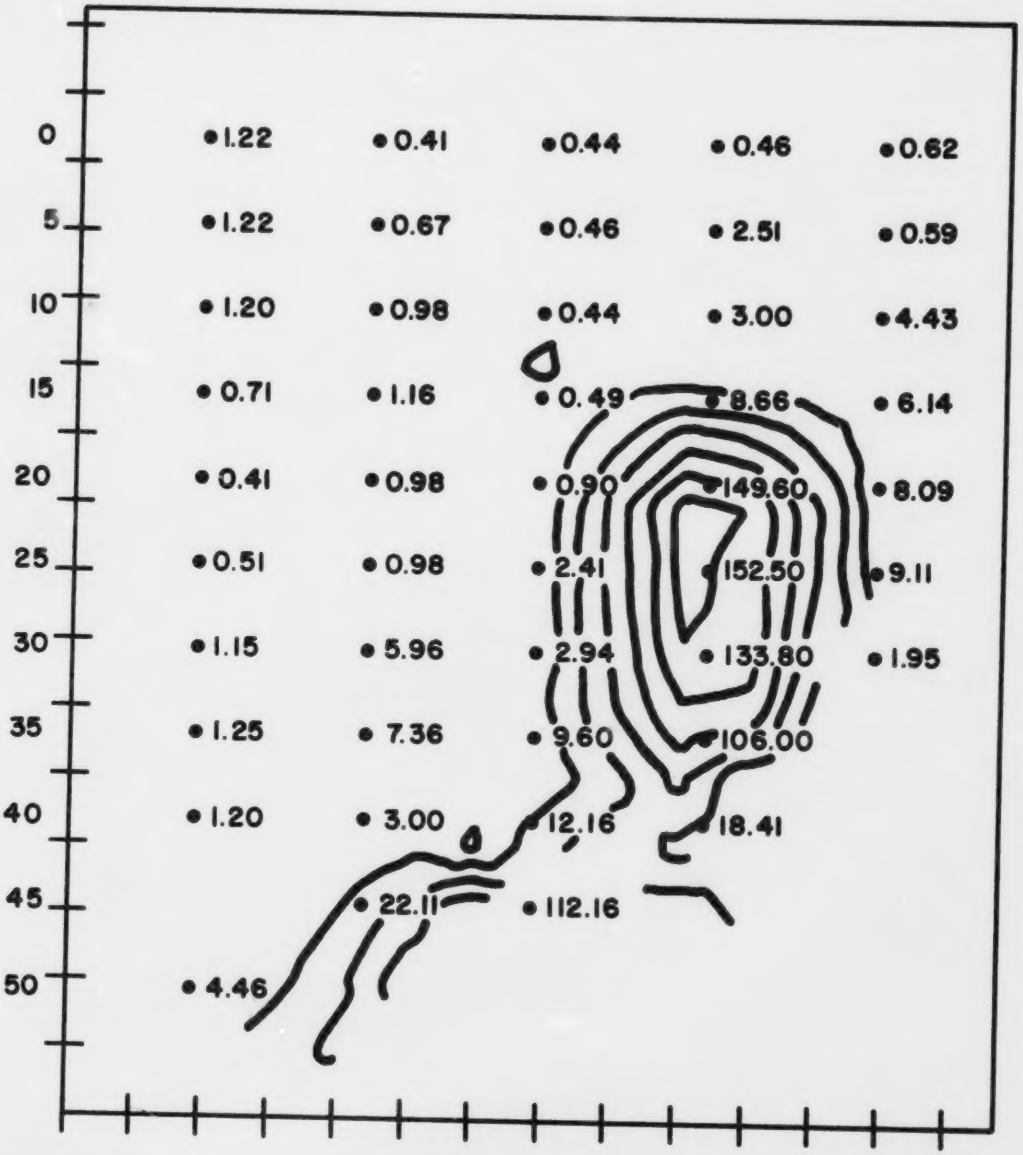


Figure 23. 93-92-91-90-89 AMMONIUM 0-50 METERS

CONTOUR INTERVAL = 20.000

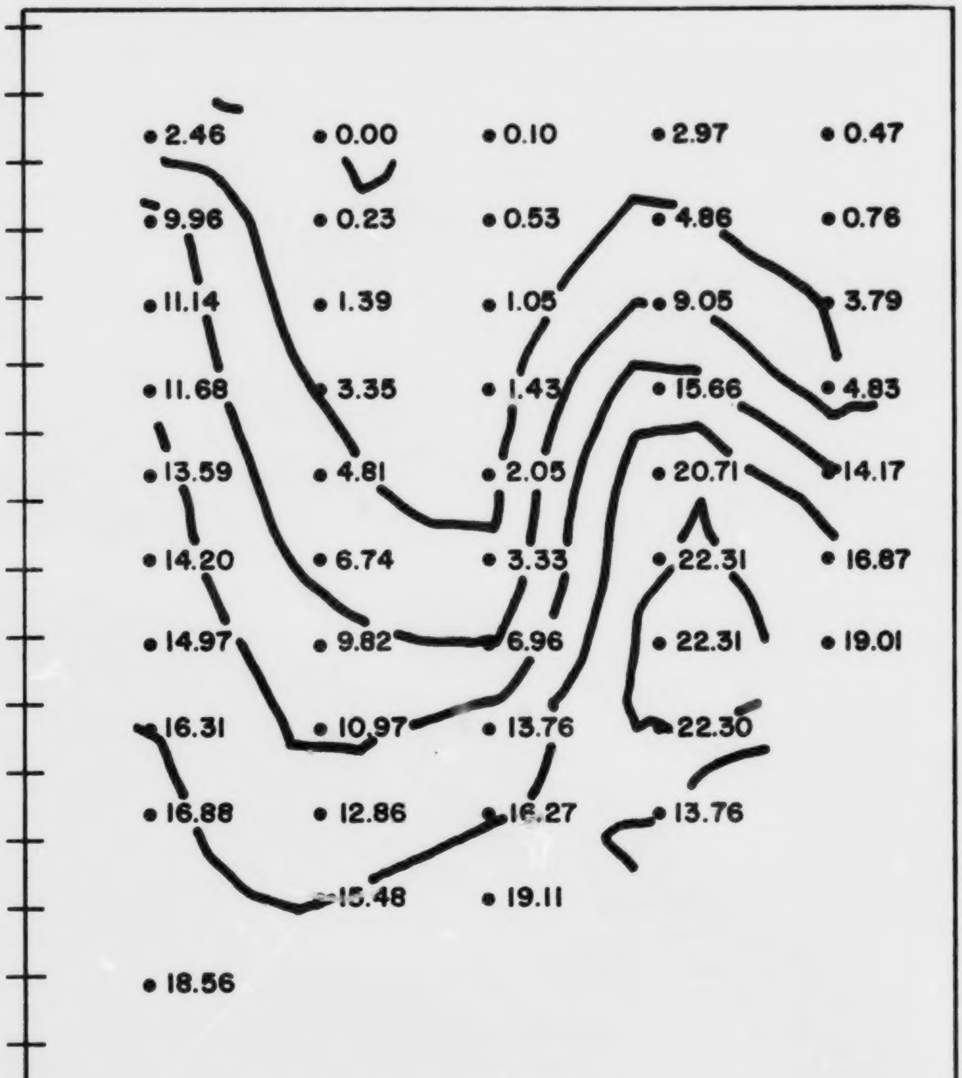
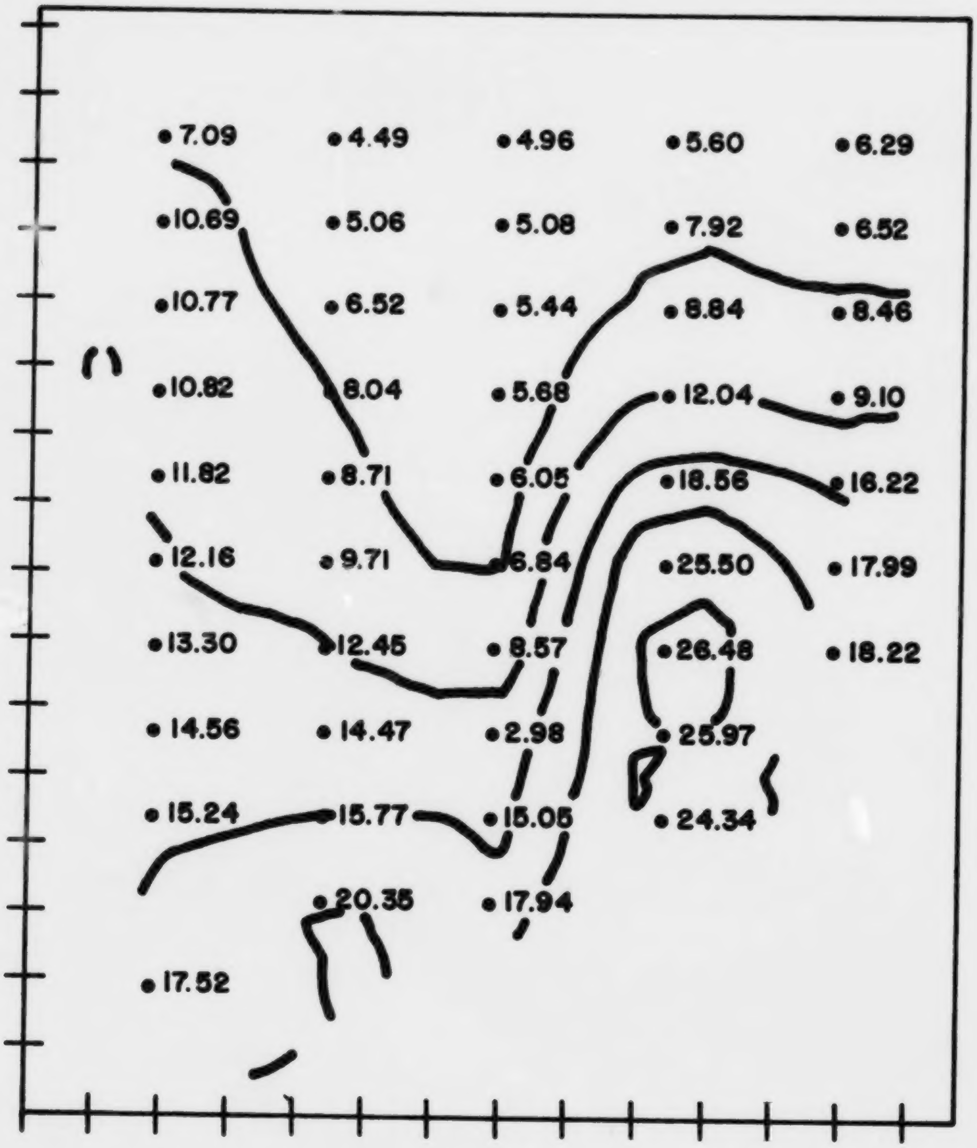

2.46
0.00
0.10
2.97
0.47
9.96
0.23
0.53
4.86
0.76
11.14
1.39
1.05
9.05
3.79
11.68
3.35
1.43
15.66
4.83
13.59
4.81
2.05
20.71
14.17
14.20
6.74
3.33
22.31
16.87
14.97
9.82
6.96
22.31
19.01
16.31
10.97
13.76
22.30
16.88
12.86
16.27
13.76
15.48
19.11
18.56

Figure 24. 93-92-91-90-89 NITRATE 0-50 METERS

CONTOUR INTERVAL = 4.000



7.09
4.49
4.96
5.60
6.29
10.69
5.06
5.08
7.92
6.52
10.77
6.52
5.44
8.84
8.46
10.82
8.04
5.68
12.04
9.10
11.82
8.71
6.05
18.56
16.22
12.16
9.71
6.84
25.50
17.99
13.30
12.45
8.57
26.48
18.22
14.56
14.47
2.98
25.97
15.24
15.77
15.05
24.34
20.35
17.94
17.52

Figure 25. 93-92-91-90-89 SILICATE 0-50 METERS

CONTOUR INTERVAL = 4.000

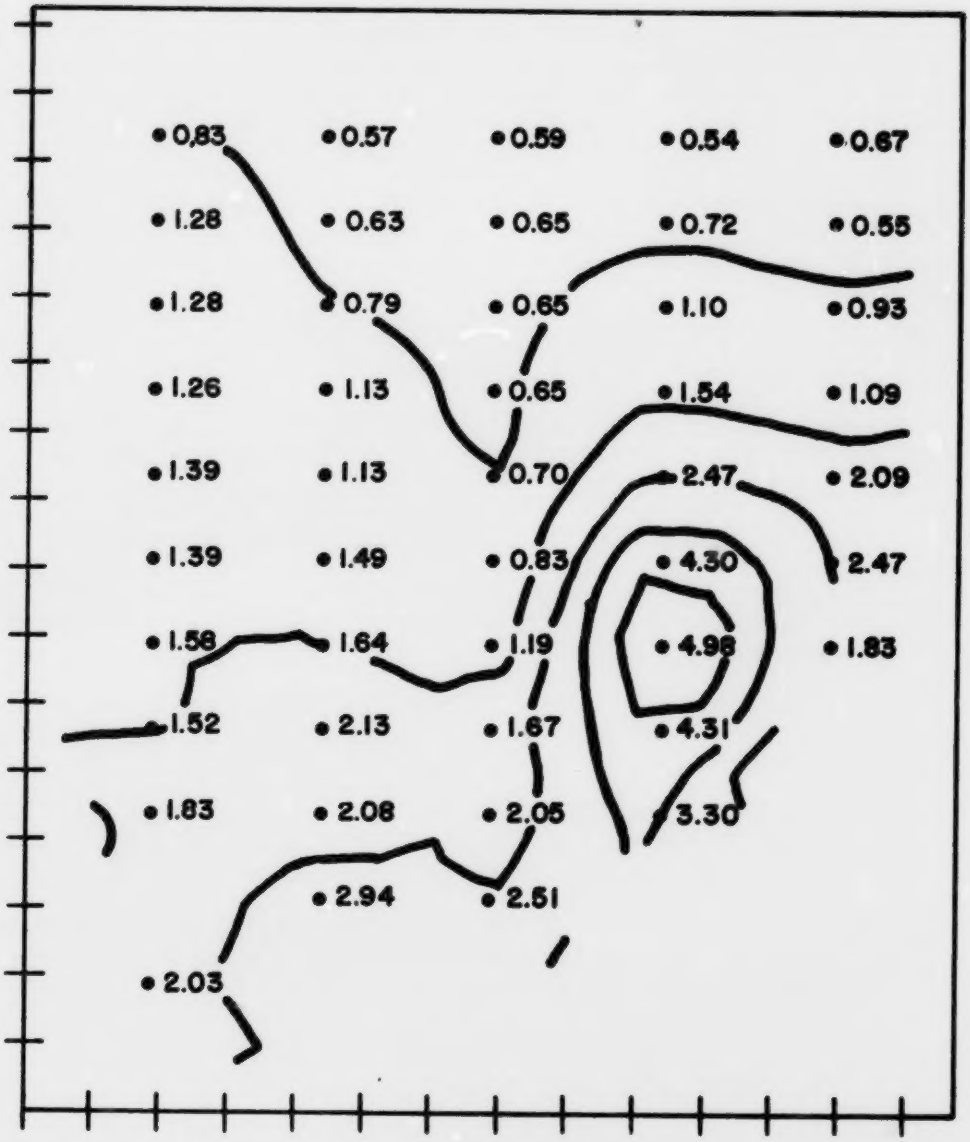

0.83
0.57
0.59
0.54
0.67
1.28
0.63
0.65
0.72
0.55
1.28
0.79
0.65
1.10
0.93
1.26
1.13
0.65
1.54
1.09
1.39
1.13
0.70
2.47
2.09
1.39
1.49
0.83
4.30
2.47
1.56
1.64
1.19
4.98
1.83
1.52
2.13
1.67
4.31
1.83
2.08
2.05
3.30
2.94
2.51
2.03

Figure 26. 93-92-91-90-89 PHOSPHATE 0-52 METERS

CONTOUR INTERVAL = 0.800

discontinuities in the temperature, salinity, silicate or phosphate fields were found.

Data collected on OUTFALL-1I showed upwelling to be an important process involved in the performance of a sewage outfall. Usually the outfalls are designed to keep the sewage field in a subsurface layer; however, complications arise when upwelling brings subsurface water to the surface. Figure 27 shows a vertical contour plot of ammonium on an offshore transect. As the sewage field approaches the coast the ammonium isopleths are displaced towards the surface. Coincident with the high ammonium, lower water temperatures were also noted at a shallow depth on the inshore stations. The increased concentration of ammonium in the euphotic zone correlated well with the large dinoflagellate bloom observed in the area.

SUMMARY

From the above considerations, a potentially effective marine pollution monitoring program should satisfy the following criteria:

1. Utilization of a multidisciplinary approach.
2. Implementation of a rigorous and sophisticated field program involving methodology for real time data acquisition and reduction.
3. Understanding the transport, interactive processes, and biological consequences of the ecosystem under investigation.
4. Development of a predictive capacity (simulation) and field validation of the theoretical framework for evaluating short and long term responses.
5. Establishment of control capability and effective water quality standards (input control) as determined by the systems investigation results.

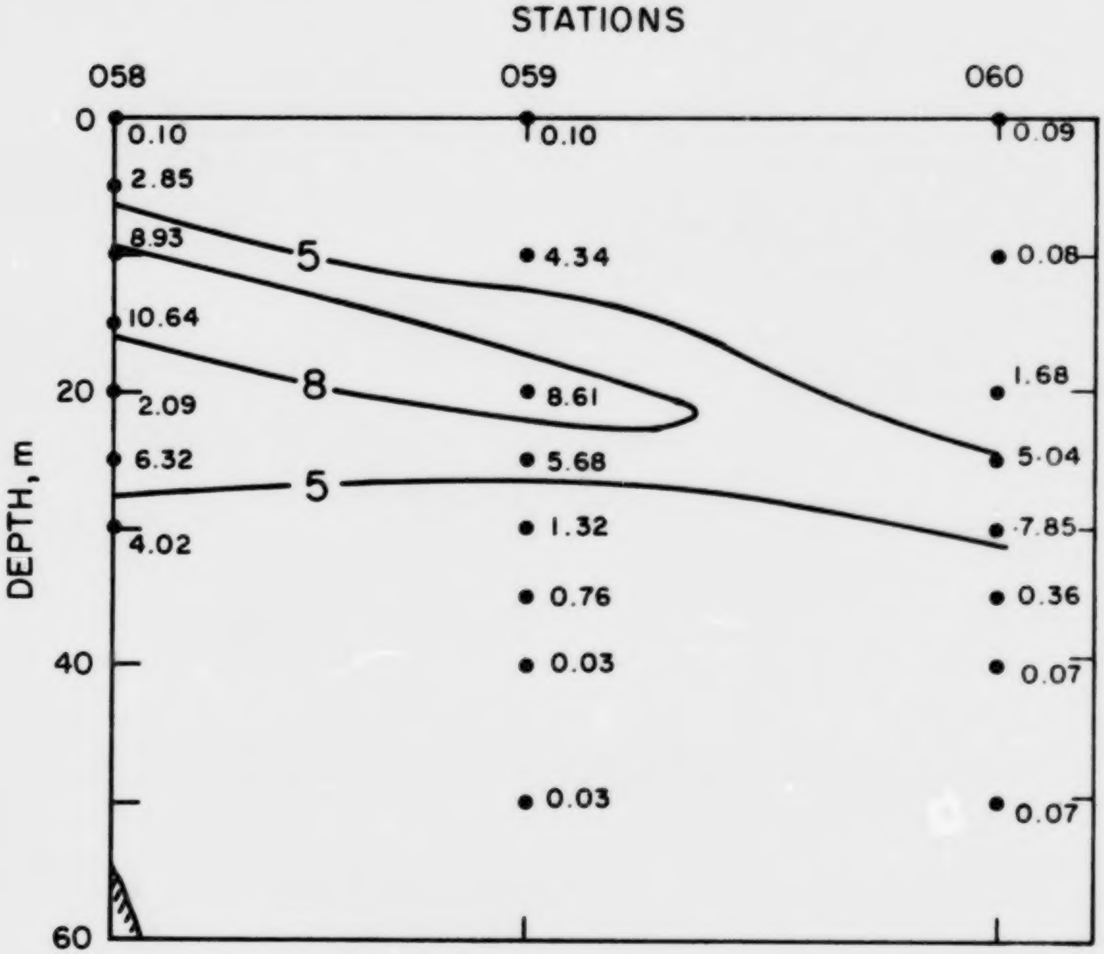

STATIONS
058
059
060
0
20
40
60
DEPTH, m
0.10
2.85
8.93
10.64
2.09
6.32
4.02
0.10
4.34
8.61
5.68
1.32
0.76
0.03
0.03
0.09
0.08
1.68
5.04
7.85
0.36
0.07
0.07
5
8
5

FIGURE 27.

METHODS AND PROBLEMS IN ANALYSIS OF PESTICIDES IN THE ESTUARINE ENVIRONEMENT

A. J. Wilson, Jr. and J. Forester
Gulf Breeze Environmental Research Laboratory,
Gulf Breeze, Florida

The presence of pesticides in the marine environment has been well documented. Cox (4) reported DDT concentrations in sea water along the Pacific Coast to range from 0.0023 parts per billion (microgram/liter) off Oregon and Washington to 0.0056 parts per billion off Southern California. Residues of DDT and dieldrin were detected in livers of fishes by Duke and Wilson (6) from the Northeastern Pacific Ocean and in gray whales by Wolman and Wilson (16). Other documentation of chlorinated hydrocarbons in the marine environment is presented by Goldberg et al. (7).

The Gulf Breeze Environmental Research Laboratory at Gulf Breeze, Florida, an associate laboratory of the National Environmental Research Center, Corvallis, Oregon, has been conducting research on the effects of pesticides in the marine environment since 1958. Since that time the laboratory has analyzed over twenty thousand samples for these pollutants in water, sediment, oysters, crabs, fish, birds and mammals. From 1965 until 1972 this facility analyzed over eight thousand samples for the National Pesticide Monitoring Program as reported by Butler (3). This report describes analytical methods employed by this Program, some recent studies in water analysis, and the need for adequate analytical quality control in marine monitoring.

NATIONAL PESTICIDE MONITORING PROGRAM

From studies at Gulf Breeze, bivalve mollusks appeared to be suitable animals to use as indicators of estuarine pollution. Adult bivalve mollusks are sessile, permitting repeated sampling of the same population. In addition, experiments by Butler (2) indicated that bivalve mollusks readily accumulate chlorinated hydrocarbons. Consequently, oysters, mussels and clams were the primary indicator organisms in this program.

Molluscs were collected at about 30-day intervals at 183 estuarine sites in 15 coastal states. Approximately 15 individuals were taken from each station by other agencies, prepared in their laboratories, and shipped to Gulf Breeze for analyses. The rationale for all analyses being conducted at Gulf Breeze was based on the premise that these methods of analyses would be consistent from station to station and month to month. This would eliminate variation in methodology and permit a more reliable interpretation of seasonal and geographic trends. In addition, it was not economically feasible at the outset to equip several satellite laboratories and conduct a suitable interlaboratory quality control program.

Prior to the start of the program several preservatives were evaluated to find a method that would allow shipment of samples without dry ice. When samples were dehydrated by mixing them with a 9:1 mixture of anhydrous sodium sulfate and Quso, a micro fine silica, they could be held at room temperature for up to 15 days without loss or degradation of tche chlorinated hydrocarbon. This procedure allowed shipment of samples in aluminum foil by surface mail from the collecting laboratory to the Gulf Breeze Laboratory.

Analytical Procedures

Molluscs were analyzed for aldrin, chlordane, o, p' and p,p' isomers of DDT and its metabolites, dieldrin, endrin heptachlor, heptachlor epoxide, lindane, methoxychlor, mirex, and toxaphene.

Sample Preparation

The tissues of 15 individuals were shucked into a one-pint Mason jar and thoroughtly homogenized with an Osterized blender. Approximately 30 g of the homogenate was added to a second Mason jar and blended with a 9:1 mixture of sodium sulfate and Quso. By alternately chilling and blending, a free-flowing powder was obtained. The blended sample was wrapped in aluminum foil and shipped to Gulf Breeze. Upon receipt of the sample, it was weighed and extracted in a Soxhlet apparatus for 4 hours with petroleum ether.

TABLE 1

PERCENTAGE RECOVERY OF PESTICIDES FROM FORTIFIED OYSTER SAMPLES

PESTICIDE	ACTUAL (PPM)	FOUND (PPM)	% RECOVERY
DDE	0.033	0.026	79
	0.033	0.026	79
	0.34	0.30	88
	0.34	0.29	85
DDD	0.067	0.061	91
	0.067	0.064	96
	0.70	0.67	96
	0.70	0.63	90
DDT	0.10	0.087	87
	0.10	0.094	94
	1.0	0.95	95
	1.0	0.92	92

Sample Clean-Up

The extracts were then purified by concentrating and transferring the extract to 250 ml separatory funnels. The extracts were diluted to 25 ml with petroleum ether and partitioned with two 50 ml portions of acetonitrile previously saturated with petroleum ether. The acetonitrile was evaporated to dryness and the residue eluted from a Florisil column, Mills et al. (11). In this technique, inceasing proportions of ethyl ether to petroleum ether were used to elute fractions containing increasingly polar insecticides.

Quantitation and Qualitation

The extracts were analyzed with Varlan Aerograph electron capture gas chromatographs. Extracts were injected into at least two 180 cm x 2 mm (ID) columns of different liquid phases. The following columns have been used: DC-200, QF-1, EGS, OV-101, mixed DC-200/QF-1, and mixed OV-101/OV-17. Liquid phases and gas chromatographic parameters were adjusted so that p,p' DDT would elute in approximately 12 minutes. The lower limit of detection for a 30 g mollusc sample was 0.010 parts per million (milligrams/per kilogram). Residues were reported on a wet weight basis without adjustment for recovery rates. Thin layer chromatography and "p" values after Bowman and Beroza (1) were used for additional confirmation of compound identity.

Extraction efficiencies were determined by re-extracting samples for longer periods of time and with different solvent systems. Recovery rates were determined by fortification of samples with known levels of pesticides. Table 1 shows typical recovery rates of DDT and its metabolites from fortified oyster samples. The values were adjusted to account for naturally-occurring DDT residues.

SEA WATER ANALYSIS

Prior to 1971, the Gulf Breeze Laboratory belonged to the Bureau of Commerical Fisheries, United States Department of Interior. During those early days, there was little known regarding the effects and kinetics of pesticides in the marine environment. Consequently, in addition to scientific publications, the laboratory frequently published quarterly and annual progress reports containing provisional or preliminary data.

In 1968 the author submitted the following report for inclusion in a Progress Report of the Bureau of Commercial Fisheries (15).

> ### STABILITY OF PESTICIDES IN SEA WATER
>
> We began preliminary studies to determine the stability of pesticides in sea water. Three p.p.b. of aldrin, p,p'-DDT, malathion, and parathion in aceton were added separately to four clear glass, one-gallon bottles containing sea water (salinity 29.8 p.p.t.; pH 8.1), one chemical per bottle. After an initial sample of the water was analyzed, the bottles were sealed and completely immersed in an outdoor flowing sea-water tank. Table 5 shows the concentration of the chemical at the indicated time interval.
>
> Although we used natural sea water in these preliminary experiments, the tests will be repeated with sterile artificial sea water so that the relative stability of the pesticides can be evaluated under standardized experimental conditions.

Because these studies showed a rapid loss of DDT in sea water, the report received a great deal of attention. As stated, these were preliminary studies but, unfortunately, amny readers carried the data beyond the scope of the experiment. Obviously, additional studies were required to account for the rapid decline of these pesticides before any conclusions could be made.

Since these studies were conducted, several investigators have reported on the transport of pesticides in marine waters. Cox (4) reported that adsorption of DDT is implicated in the uptake mechanism for algal cells. His experiments also indicate that particules less than 1-2 μ diameter carry most of the DDT residues in whole sea water. Working in the laboratory with six species of marine algae, Rice and Sikka (13) found that all species concentrated DDT to levels many times higher than the original concentration of the medium. Transformation of DDT and cyclodience

Table 5.--Stability of pesticides in natural sea water (salinity 29.8 p.p.t.; pH 8.1)

Pesticide	Days after start of experiment					
	0	6	17	24	31	38
	P.p.b.	P.p.b.	P.p.b.	P.p.b.	P.p.b.	P.p.b.
p,p'-DDT.....	2.9	.75	1.0	.27	.18	.16
p,p'-DDE*..		.096	.95	.065	.034	.037
p,p'-DDD*..			.081	.041	.038	.037
Aldrin**.....	2.6	.58	.096	<0.01	<0.01	<0.01
Dieldrin*..		.74	1.0	1.0	.75	.56
Malathion....	3.0	<0.2	<0.2	--	—	—
Parathion....	2.9	1.9	1.25	1.0	.71	.37

*Metabolites of parent compound.

**From the seventeenth day onward, 2 unidentified peaks appeared on the chromatographic charts after aldrin had eluted.

insecticide took place in surface films, plankton, and algae but not in water from the open ocean according to Patil *et al*. (12).

Recently, experiments have been conducted to determine the cause of loss of DDT in the 1968 studies at Gulf Breeze. The experiments were repeated under similar conditions with the exception that duplicate samples were analyzed. In 1968 the salinity was 29.8 ppt (parts per thousand) and the incubation temperature averaged 29 C; in 1973, the salinity was 24.0 ppt and the incubation temperature averaged 12 C. Figure 1 shows the percentage recovery of DDT (including DDE and DDD) during the two experiments. The results are similar except for the 17 day 1968 analysis.

Experiments were then performed to determine if DDT was adsorbed to the walls of the test containers. Additional experiments were designed to determine if DDT was converted to DDA, a water soluble metabolite of DDT, which would not have been detected by the method of analyses used in the initial study. These experiments showed that less than 1% of the DDT was adsorbed to the walls of the glass bottles andd furthermore that there was no conversion to DDA.

Since petroleum ether was the solvent used for extracting the DDT from sea water, the following studies were initiated to evaluate the extraction efficienceies of other solvent systems. Duplicate one-gallon bottles of clear glass, containing 3.5 liters of sea water or distilled water, were fortified with 10.5 μg of p,p' DDT in 350 $\mu\ell$ of acetone to yield a concentration 3.0 ppb. Duplicate 500 ml samples were taken from each bottle and extracted with one of the following solvents: three 50 ml portions of petroleum ether, two 50 ml portions of 15% ethyl ether in hexane followed by one 50 ml portion of hexane, or three 50 ml portions of methylene chloride. All solvents were dried with sodium sulfate, concentrated to an appropriate volume and analyzed by electron capture gas chromatography. Just prior to extraction, all samples were fortified with o,p'DDE to evaluate the integrity of the analyses. The recovery rate of o,p'DDE in all tests was greater than 89%, indicating no significant loss during analyses.

After initial sampling, the bottles were sealed and incubated at 20 C under controlled light conditions (12 hours light, 12 hours dark). Duplicate samples of 500 ml were extracted at various time intervals.

Tables 2-5 show the average percentage recovery of p,p'DDT extracted from duplicate sea water or distilled

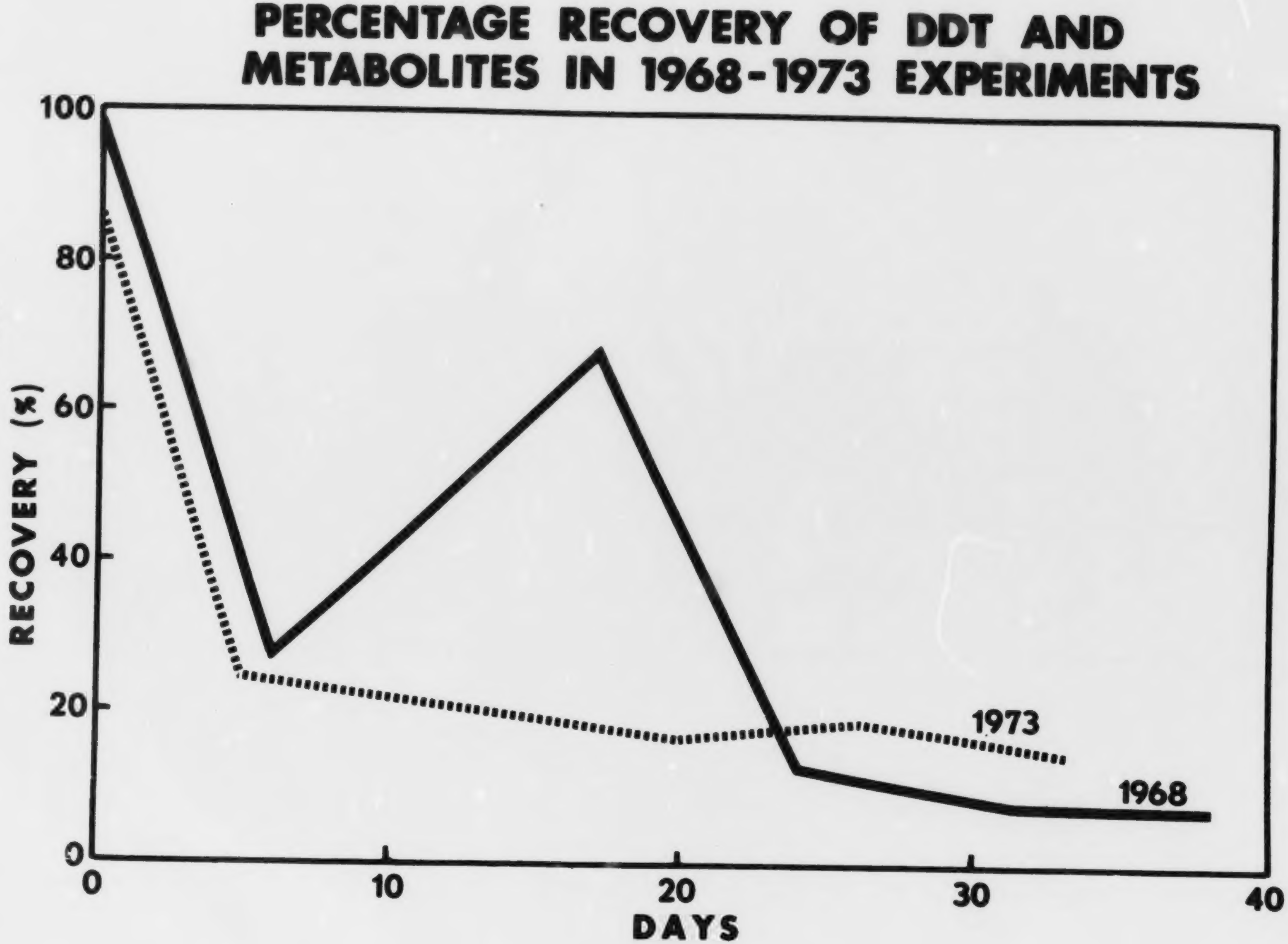


Figure 1. Percentage Recovery of DDT and Metabolites in 1968 and 1973 Experiments

TABLE 2

PERCENTAGE RECOVERY OF P,P' DDT FROM SEA WATER BY DIFFERENT EXTRACTION SOLVENTS

DAY	EXTRACTION SOLVENT		
	PETROLEUM ETHER	15% ETHYL ETHER IN HEXANE	METHYLENE CHLORIDE
0	93	93	93
6	67	66	76

TABLE 3

PERCENTAGE RECOVERY OF P,P' DDT FROM SEA WATER BY PETROLEUM ETHER AND METHYLENE CHLORIDE

DAY	PETROLEUM ETHER	METHYLENE CHLORIDE
0	90	95
4	67	85

TABLE 4

PERCENTAGE RECOVERY OF P,P' DDT FROM SEA WATER AND DISTILLED WATER BY PETROLEUM ETHER AND METHYLENE CHLORIDE

DAY	SEA WATER		DISTILLED WATER	
	PETROLEUM ETHER	METHYLENE CHLORIDE	PETROLEUM ETHER	METHYLENE CHLORIDE
0	90	94	90	91
7	58	78	90	91
14	46	68	94	92

TABLE 5

PERCENTAGE RECOVERY OF P,P'DDT FROM SEA WATER INCUBATED UNDER DIFFERENT LIGHT AND TEMPERATURE CONDITIONS

DAY	12 HOUR LIGHT AND 12 HOUR DARK AT 20 C	DARK AT 5 C
0	87	88
7	69	81
14	68	86

water samples up to 14 days after initiation of the experiment. p,p'DDE was the only metabolite measured, and since it never exceeded 2% of the parent compound it is not included in the percentage recoveries. The sea water was collected adjacent to the Gulf Breeze Laboratory in Santa Rosa Sound and the salinity ranged from 16 ppt to 21 ppt.

Table 2 shows that immediately after the sea water (21 ppt) was fortified with 3.0 ppb of DDT all solvent systems removed 93% of the DDT. After six days of incubation this level of recovery was not observed with any of the solvents tested. However, methylene chloride was more efficient than petroleum ether or 15% ethyl in hexane. Part of this experiment was repeated with sea water (16 ppt) and incubated for 4 days with similar results (Table 3).

An experiment was performed with sea water (21 ppt) and distilled water using petroleum ether and methylene chloride. Table 4 shows that immediately after fortification, recoveries were greater than 90% for water and solvents.

After 14 days, similar recoveries were observed only in distilled water. In sea water however, there was 49% and 28% reduction in recovery with petroleum ether and methylene chloride respectively. Since distilled water is devoid of particulate matter, this study suggests that DDT may be absorbed or adsorbed to plankton or particulate matter in sea water and the sorbed material was not removed resulting in low recoveries of DDT. This would explain the initially high extraction efficiency of DDT followed by the decline in recovery as DDT was associated with the particulate phase. Since methylene chloride was the most polar solvent used, it would have a greater affinity for removing the sorbed DDT.

In another test, duplicate bottles containing sea water (20 ppt) and DDT were incubated under controlled lighting conditions at 20 C and another set incubated at 5 C without light. Both were extracted with methylene chloride at various time intervals. Table 5 shows low recovery at 14 days under controlled lighting condition. However, those samples incubated at 5 C in total darkness did not show a significant decrease in recovery rate. Since the metabolic activity of plankton was probably inhibited under these temperatures and lighting conditions, these results suggest that DDT may be absorbed rather than adsorbed by plankton. However, Rice and Sikka (13) comparing the uptake of DDT by living and dead algae found that cells accumulated equal amounts of the pesticide.

Interaction of pesticides between water and particulate matter are complex. Not only do light and temperature appear to alter equilibria, but other physical and chemical factors have effects. Evaluating liquid-liquid extraction techniques of herbicides from river water, Suffet (14) observed that the isopropyl ester of 2,4-D was adsorbed to particulate matter in river water and that the amount changed by alteration of the pH of the water. Huang and Liao (10) found that adsorption of DDT to clays was rapid but the amount differed with the type of clay. A mixed culture of algae consisting mainly of Vauchenia had a greater adsorption for DDT than bentonite according to Hill and McCarty (9). Cox (5) reported that in natural marine populations virtually all of the DDT available for uptake was incorporated onto phytoplankton, but this may only account for 10% of the DDT residues recoverable from whole sea water.

These experiments support the work of other investigators in that DDT and other pesticides are extremely hydrophobic and can easily be adsorbed or absorbed by suspended matter from liquid solutions. The 1968 experiments at Gulf Breeze supports the concept that physical or chemical transformations of pesticides altered the extraction efficiences of the solvent and prevented complete recovery of the compounds. Obviously, additional work needs to be done to account for all of the chemical added to the test system.

It is difficult to relate laboratory findings directly to these of the estuary or open oceans. However, the laboratory data illustrate clearly some problems that could be encountered in monitoring sea water for pesticide pollution. The conventional analyses of water samples by liquid-liquid extraction techniques may provide invalid data if suspended matter is not considered. Standardized methods are needed to analyze the water column and suspended material separately.

Recently, a synthetic resin, Amberlite XAD-2 was evaluated as an adsorption medium for chlorinated hydrocarbons dissolved in sea water by Harvey (8). This technique utilizes large volumes of sea water and therefore permits greater sensitivity in analysis. In addition, the method eliminates the problems encountered in transport of large samples of water. Pollutants could be adsorbed on the resin at the sampling site and shipped to the appropriate analytical laboratory for desorption. Since the resin only removes the dissolved portion, additional samplings would be required to determine the levels absorbed or adsorbed to particulate matter.

ANALYTICAL QUALITY CONTROL

All pesticide residue laboratories should maintain an adequate analytical quality control program. The program should include both an intra-laboratory performance evaluation of personnel and methodology and an inter-laboratory sample exchange program. These programs are time consuming but are essential to the generation of valid analytical data.

Table 1 shows the recovery rates of oyster samples fortified with known concentrations of pesticides. Fortification techniques provide data only on the recovery efficiency of the total analytical procedure and not on the extraction step. Field residues may be subject to physical and chemical transformation and therefore may not be in the same physical or chemical state as the fortified sample. Table 2 illustrates the errors that can result from fortified samples. Extraction of water samples immediately after fortification yields relatively high recovery efficiency with all solvent systems in sea water. Analyses several days later show the relative inefficienceis of the solvents systems used in sea water. Regardless of the analytical method used or the substrate being extracted, recovery data must be obtained on the extraction efficiency and the total analytical procedure.

There are several other factors in residue laboratories which, if ignored, may lead to inaccurate data. To name a few: (a) all glassware must be clean and free of residues; (b) the purity of all reagents used during analyses must be determined; (c) the accuracy of analytical standards must be maintained; (d) the condition of all components of the gas chromatograph must be optimized; and (e) laboratory personnel should be thoroughly trained.

An area that needs further study is the use of internal standards in marine pesticide monitoring. Currently, the Gulf Breeze Laboratory is no longer affiliated with a large monitoring program. Almost all samples submitted for analysis are of known identity. Most of these samples are fortified with an internal standard, just prior to analysis. The standard is usually a compound which behaves in an analytically similar way to the compound of interest. This technique is valuable in assessing the validity of the analysis. This same technique could also be applied to marine monitoring samples if an appropriate compound could be found that would not interfere with the monitored pesticides. It would be extremely valuable in laboratories with large sample volumes where close supervision of laboratory operations is not possible.

The analysis of marine samples for chlorinated hydrocarbon pesticides is at times complicated by the presence of polychlorinated biphenyl compounds (PCB). These compounds are industrial pollutants and are produced in the United States under the trade name Aroclor. They have chromatograph retention times similar to the organochlorine pesticides and therefore complicate the analysis when both are present in a sample. Several techniques have been described for the separation of PCB from organochlorine pesticides. A review of these methods were presented by Zitko and Choi (17). These techniques are time consuming and, in general, semiquantitative. In addition, differential absorption or metabolism of the Aroclor isomers in marine biota prevent accurate analysis of the PCB's. In view of these facts and the large number of samples that could result from a global monitoring program, this area of analysis required further study and/or standardization.

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BIOLOGICAL PROBLEMS IN ESTUARINE MONITORING

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INTRODUCTION

Awareness of the extent of persistent organochlorine pollution both in the physical environment and the biota has made apparent the need for continuing surveillance programs to assess the problems. During the period 1965-72, samples collected in the National Estuaries Monitoring Program, as well as in other studies, revealed some of the difficulties involved in the interpretation of residue data. Bivalve molluscs are efficient bioassay tools for identifying the ebb and flow of pollutants in surrounding waters, and the monthly monitoring of molluscan populations made obvious the dynamic nature of organochlorine pollution in the estuary. The image of polluted versus unpolluted estuaries was soon modified by monitoring data that indicated instead the movement of relatively discrete masses of clean and polluted water through the estuary. Organochlorine residues in molluscs fluctuated from month to month in response to the sometimes transitory nature of the pollution and contrasted sharply at times with residues observed in other elements of the associated biota.

To gain increased understanding of the significance of residue levels, sample collections in problem areas were intensified in variety, frequency, and size; and additional work was undertaken under laboratory conditions. The experimental program demonstrated that the uptake and retention of persistent residues varied unpredictably with the environmental element sampled. It became clear that surveillance or monitoring systems had to be carefully designed if they were to provide answers to specific program objectives.

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A primary concern is the extent of environmental degradation - with the implied intention of reversing or at least halting harmful pollution trends. But individual agency goals are much more specific. For expedience and because of monetary restrictions, programs are usually designed to clarify rather narrowly defined sectors of the whole pollution picture. Often, the foremost question concerns only the existence of a human health problem, and because of some environmental pollution problems may go uninvestigated. The idea that any changes harmful to the environment will eventually affect man is still not generally accepted.

The basic needs for environmental surveillance require programs designed to provide current data that will be adequate to identify later deleterious changes. Monitoring data should indicate existing problems as well as their extent in time and space. To provide information of this type, protocols must take into account the influence of various physiological and ecological factors affecting the sample selected for study. Some of the anomalous residues data acquired so far are understandable, but reasons for others are less certain. The following discussion of factors affecting persistent organochlorine residues indicates some of the options available in selecting the most informative sample types.

FIELD AND LABORATORY OBSERVATIONS

Residue differentials resulting from kind of species monitored:

The necessity for utilizating different species to monitor pesticides in different coastal areas prompted the conduct of laboratory experiments to determine the relative sensitivity of molluscan species selected for their diversity in salinity tolerance. A number of controlled experiments have shown the relative uniformity of DDT residue formation in the eastern oyster (*Crassostrea virginica*) under varying estuarine conditions of salinity and temperature. Observations of four other molluscan species exposed simultaneously to a mixture of common organochlorine pesticides indicated considerable variation in the relative rates at which residues were acquired, then lost when clean water was restored to them (Table 1) (3).

Studies were directed toward evaluating residue flushing rates in the hard clam (*Mercenaria mercenaria*) because of

Bioassay animal	Magnification in Body after 5 days exposure	Percentage loss after 7 days in clean water
Soft clam (*Mya arenaria*)	3000	74
Eastern oyster (*Crassostrea virginica*)	1200	50
Marsh clam (*Rangia cuneata*)	700	50
Asiatic clam (*Corbicula fluminea*)	600	30
Hard clam (*Mercenaria mercenaria*)	500	75

Table 1. Average biomagnification and depuration rates of a of seven common chlorinated pesticides by molluscs exposed in a flowing seawater system.

the prevalence of this species in the New England area and its observed poor performance in storing DDT residues. The biological half-life of pesticide residues in molluscs under the same conditions is of importance in determining the movement of pollutants in the estuary. Clams and oysters with DDT residues of about 40 ppm were placed in aquaria with uncontaminated flowing seawater. The clams flushed out 50 percent of their DDT residue within five days. At the end of 15 days less than 2% of the original DDT burden remained. In contrast, the oysters still contained 50% of the residue at the end of 15 days and significant amounts of DDT were present after a month. Clearly, clams would have to be sampled more frequently than oysters to determine pollution inputs.

Uptake and depuration rates in molluscs under laboratory conditions must be accepted as relative figures and extrapolated to field conditions with caution. In one study, for example, oysters exposed to mercury accumulated residues of about 28 ppm (8). Relatively small declines in residues took place in the following 160 days of depuration. The authors concluded from the data that mercury residues acquired by oysters in nature might require years to decline to acceptable levels. Their experimental conditions were described as a 'natural system' but the oysters were supplied only 2 ℓ clean water/animal per hour. This volume of water should be compared to their well-known utilization of up to 30 ℓ per hour even under experimental conditions (11). It is probable that in this experiment, the mercury was continuously recycled and the oysters never had opportunity to adequately flush their tissues.

The mercury exposure experiment is perhaps comparable to a situation monitored in Mecox Bay, Long Island (6). The water of this bay are typically isolated from the ocean by sand bars, and only periodically do winter storms cut the bars and permit flushing (10). In the period 1966-72, the oysters in this bay were continucusly contaminated with DDT and at a maximum level higher than that observed in any of the other 16 estuarine stations monitored in the New York despite apparently low use records of DDT in the area. In my opinion, these oysters were continuously contaminated with recycled DDT because of the inadequate supply of clean water.

Species differentials in pesticide uptake are apparent in populations monitored in Conscience Bay, New York (6). During the first half of 1968, monthly mussel samples (Mytilus edulis) from this bay contained about 50 ppb of DDT and its metabolites. In the latter half of the year and until April 1969, hard clam was substituted because it was easier to collect. During this period no DDT residues were detected. In April and thereafter, the mussel was again utilized and DDT residues were found to be once more 50 ppb or higher. In this case, the convenience of collecting hard clam samples 'determined' the presence or absence of DDT pollution in the bay.

Similar discrepancies were observed in simultaneous collections of spot (Leiostomus xanthurus) and oysters in a South Carolina estuary in 1968. Residues of DDT occurred in all monthly samples of the fish and levels fluctuated between 100 and 300 ppb although the fish sampled were of uniform size. Oyster samples showed DDT pollution was present only during the first six months of the year. If

DDT pollution was present after that, it was not stored by the oyster at levels chemically detectable. Numerous other studies have demonstrated the retention and gradual increase of organochlorine residues in fish, at least until their first spawning period. Consequently, it is not possible to determine from periodic fish analyses the seasonal occurrence of DDT pollution in an estuary.

Unexpected variations occur in the sensitivity or selectivity of the biota in the same ecosystem to organochlorine pollution even though the species monitored are presumed to occupy similar tropic levels. These differences are well-illustrated in a study of monitoring methods conducted in Virginia (12). Sample analyses demonstrated the existence of residues of three different polychlorinated biphenyl compounds (Arcclors 1242, 1254, and 1260) in different types of samples. All samples were large enough to minimize individual variation. In March, Aroclor 1242 was detected only in anchovies (*Anchoa* sp.); Aroclor 1254 was present in silversides (*Menidia* sp.), oysters and two series of plankton tows. A third series of plankton samples contained only Arcclor 1260. Three months later, Aroclor 1242 was present in both anchovies and silversides while Aroclor 1254 was no longer found in silversides but was still present in oysters and plankton. Aroclor 1260 was not detected. These variations in residue accumulations in a short period in one river system are not easily explained (Table 2).

	March	June
Aroclor 1242	Fish A 1/ ------	Fish A Fish S
Aroclor 1254	Fish S 2/ Oysters Plankton Sediment	------ Oysters Plankton Sediment
Aroclor 1260	Plankton	------

1/ Anchovies
2/ Silversides

Table 2. Estuarine samples containing residues of PCB's at two sampling periods

Past dependence on fortuitous samples (found dead, happened to be caught in a net, etc.) to assess pollution levels has resulted in an uneven if not confused picture of general environmental contamination. Broad community studies frequently show persistent organochlorine residues differing by an order of magnitude in species of similar habit (9, 20). Consequently, valid assessment of persistent residues in any one species of a community requires not only an understanding of its position in the trophic structure but also its variability as compared to similar species.

Residue differentials resulting from age of individuals monitored.

In fish and other vertebrates, the localization of organochlorines is highly lipid tissues and their persistence, at least in part, for long periods is well-documented. For example, DDT tends to accumulate gradually in pinfish (Lagodon rhomboides) and an approximately tenfold increase from 0-to-1-year fish has been documented (14). There is an approximate doubling of DDT residues in lake trout in the Great Lakes each year up to age 10 (17). Accumulation of dieldrin residues in these fish are proportional to but age occurs at lower magnitude. Approximate doubling of mercury levels during the first few years has been reported in salmon (Salmo salar) from rivers in Sweden (18).

In contrast, organochlorine residues in molluscs do not persist from year to year in the absence of pollution despite their affinity for lipid tissues. Residue levels fluctuate widely depending on the input of pollutants to the estuarine system and not on the age of the mollusc. Residue levels can be correlated frequently with pesticide usage in the drainage system when oysters of similar size are monitored. However, oysters of different size do react differentially to similar pollutant levels and, in general, residues per gram of tissue are higher in small oysters than in oysters of significantly larger size. Although the data are not at hand, I assume that this difference is a function of the larger amount of water circulated (filtered) per gram of tissue in small oysters compared to large ones. This higher biological magnification of residues in small oysters might be disasterous since, with a given amount of contaminated tissue consumed, predators would ingest much more DDT from small as opposed to large oysters.

This demonstrated increase in persistent residues with age complicates interpretation of data in many instances where the age of individuals making up the sample are unavailable and difficult to ascertain.

Residue differentials resulting from variations among individuals.

For obvious reasons, uniform populations or aggregates of individuals are selected as often as possible as indicators of environmental degradation. But both laboratory and field studies demonstrate that there can be much individual variation in levels of organochlorine residues. DDT residue levels in a presumably uniform sample of yearling pinfish in a Florida estuary ranged from 13.7 ppm to 0.5 ppm (14). The standard error in these data was more than 230% of the arithmetic mean. The average in two of the fish was 13.2 ppm while the average residue in the other eight fish was only 1.5 ppm DDT. Not all groups analyzed have shown this diversity but it must be anticipated in data interpretations. Diversity in residue levels of molluscan populations is less extreme and may be illustrated by one group of mature oysters exposed to DDT under controlled conditions in flowing seawater for 96 hours. In the 10 individuals, total DDT residues ranged from about 4 to 25 ppm with a mean value of 11.6 ppm. In this case, the standard deviation was only about 50% of the mean.

Much larger samples must be collected to obtain more uniform data, but this is a costly and time-consuming process. We undertook in 1971 as exploratory project to examine the merits of larger sample size (12). Oysters, two species of fish, plankton and sediment cores were collected concurrently at two stations in two Virginia rivers at a two-month interval. Ten samples of 15 oysters and 10 samples of 25 fish were collected at each station. In general, PCB's were the principal residues found and the spread of values was reasonably small. Standard errors, for example, were about 15 to 30% of the arithmetic means. Data on sediment cores were less uniform even though samples were collected in a restricted area. In one series, for example, only 3 of 10 cores had measurable residues of PCB. This means that had only replicate cores been taken there would have been about a 50-50 chance that one of the cores would have contained a residue. These data are significant in light of the fact that sometimes only a single sediment core is collected to assess pollution in an entire estuary.

Numerous studies have shown that the magnitude of persistent residue levels in sediments is usually inversely proportional to grain size. In consequence, care must be exercised in selecting representative sample sites, as well as in analyzing an adequate number of samples.

Despite the mathematical pleasure in achieving uniformity in sampling results, it must be emphasized that averaging data, either in the electric blender or the calculator, may lead to serious management errors. Animals, in general, are not responsive to average pollution levels, they survive and flourish as a result of environmental extremes. A single high incidence of endrin in the environment can be satisfactorily averaged away on paper, but at the time of its occurrence all of the endemic animals may have been killed.

There is a further important consideration in assessing the importance of organochlorine residues in aquatic biota. It is axiomatic that, with a given level of environmental pollution, the sensitive species and the sensitive individuals of more tolerant species will be affected first. In one experiment, pinfish were fed a diet contaminated with about 4 ppm of DDT (4). At the end of the 14th day, the ten surviving fish were sacrificed and found to have average residues of about 4 ppm. The 25 fish dying during the 2-week period averaged about 0.6 ppm of DDT, less than 1/6 as much as the resistant individuals. Obviously, the magnitude of residues occurring in apparently healthy populations is not necessarily an indication of tolerable pollution levels.

Residue differentials showing seasonal variations.

Monitoring programs and laboratory studies in which periodic samples have been collected at sufficiently short intervals may show clearly defined seasonal variations in residue levels. Such cyclic changes in the presence of relatively constant pollution loading are indicative of basis physiological changes in the monitored species. For example, studies of speckled seatrout in Texas showed a 75% decrease in gonad DDT residues in mature fish in the late fall (7). Lowe reported a more than 50% decline in DDT and a 70% decline in toxaphene residues in oysters continuously exposed to these pollutants (15). Oysters exposed simultaneouly to PCB, dieldrin and DDT in the laboratory lost from 45 to 80% of the residues within a short period (16). Whole body residues of mercury in oysters are also reported to decline seasonally in a manner similar to the organochlorine compounds (8).

In each case, the abrupt declines have been clearly identified with the normal spawning period of the animal in question. That there should be a significant percentage loss of such residues on a seasonal basis is entirely predictable in view of the localization, for example, of DDT oyster gametes (1).

Seasonal declines in levels of organochlorine residues in oyster are also clearly associated with fluctuating levels in the input of pollutants into the aquatic system. In southwest Florida, the former agricultural use of DDT was intensified just prior to the harvest of sweet corn and sugar cane. Residue levels of DDT in oysters collected monthly in an associated river basin reflected this management practice; peak DDT residues in oysters were as much as 1000x higher than minimal residue levels in 1967-68 (6).

Organochlorine residue patterns in estuarine biota resulting from agricultural and industrial practices may be masked by the effects of marked changes in river discharge in the drainage basin because of rainfall variations.

Residue differentials affected by body region monitored.

Regardless of the mode of entry of organochlorine into living tissues, there is a partitioning and partial immobilization of these compounds in fatty tissues because of their lipophilic nature. Such segregation is clearly demonstrated in fish in which adipose tissues are highly localized. In coho salmon, for example, DDT residues in a mid-body fillet range from an average of about 65 ppm in fatty tissues to less than 6 ppm in the muscle. The whole body DDT residue of similar fish was about 12 ppm (17).

As discussed above, residue levels increase with age in fish. In aquatic mammals the localization of residues in older individuals may be even more striking. In a dead porpoise (Tursiops truncatus) found near Pensacola, Florida, total DDT residues ranged from about 1.5 ppm in blood, 7 ppm in the muscle, 9 ppm in the brain to 33 ppm in the liver, and more than 500 ppm in the blubber (13). Even in oysters, where total body fat is only about 4%, there is a marked localization of DDT residues in the digestive gland, and, seasonally, in the gonad which contains more fat than other organs.

In general monitoring or surveillance programs, the localization of persistent residues in particular body regions has little importance from the point of view of either human health or resource protection so long as the monitored species is small enough to be analyzed on a whole-body basis. Residues large enough to warrant further investigation will show up in such analyses regardless of their location in the body.

It is quite another matter, however, if as in market-basket surveillance programs, only the products, e.g., lobster and shrimp tails or tuna muscle, are examined. It is conceivable then the edible portions would contain negligible organochlorine residues while the discarded body parts could contain amounts detrimental to the productivity of the animal itself or to other animals preying on it in nature.

SUMMARY

Successful monitoring of the estuarine environment for persistent organochlorine pollutants is dependent in large measure on the collection of appropriately biased samples. Statistically randomized sample collections are unsatisfactory for the simple reason that pollution patterns are not random. The transport of persistent residues are dependent on a large number of biological factors which in turn are modified by physical and chemical parameters of the environment.

Monitoring programs have several basic functions and requirements. First, they should record existing residues of persistent pollutants that occur at significant trophic levels in aquatic ecosystems. Sample collection protocols, as well as analytical procedures, must be sufficiently standardized to ensure the comparability of data, not only from one area to another but also from year to year. It is essential that monitoring programs collect comparable data for sufficiently long periods of time so that pollution trends can be identified. Finally, it should be stressed that monitoring data must be transmitted on a timely basis to action agencies. Agencies mandated to identify and regulate pollution sources, agencies with resource protection responsibilities, and agencies concerned with human welfare must have clearly established communications channels with environmental monitoring programs.

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DETERMINATION OF METALS IN SEA WATER

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1. INTRODUCTION

Pollution by heavy metals in the marine environment can occur from waste dumping, direct wastewater discharge, and from flow of polluted rivers. The precipitating action by sea water salts on waste colloidal material should sediment significant portions of the metals. The sheer volume of sea water will greatly dilute the remaining portion. Nevertheless, constant dumping or discharge of metals in fixed regions over a long period of time can only be detrimental.

Natural sea water is a complex mixture of virtually every chemical element. It differs from fresh water by having a much higher dissolved salt content. The salt matrix causes interference problems with nearly all conventional fresh water metal analytical methods. The normal composition of individual metals in marine waters is less than 20 g/l (ppb). Localized pollution can increase the level many fold. The analyst must give much effort and attention to get consistent and reliable results for such low concentrations.

2. METALLIC FORMS

The chemical forms of metals in sea water can be divided into six fractions (Table I). They are inorganic particulate, organic particulate, particle adsorbed, inorganic complexed, organic complexed, and ionic.

The complexed and ionic can be classified as dissolved. A method of analysis may detect all or only part of the forms present. The fraction that responds will be dependent on sample pretreatment and the analytical technique used. For example, filtration should separate particulates from dissolved fractions. Ion exchange resins may perform a complete metal separation by retaining all fractions either

TABLE I

METALLIC FORMS IN SEAWATER

Fraction	Example
Inorganic Particulate	Me S
Organic Particulate	Me In Plant Life
Particle Adsorbed	Me^+ On Colloidal Material
Inorganic Complexed	$MeCl^{+-}$
Organic Complexed	Me-humates
Ionic	Me^+

by chelation or particle entrapment. Chemical forms of metals in sea water are variables that must be considered when interpreting the analytical measurement.

3. SAMPLE PREPARATION

Great care is required during collection and preparation of samples for them to be representative of a specific marine locality. Metals loss or contamination in sample containers, by chemical changes and by impure reagents, are potential problems that need be controlled.

Most trace metal analytical methods are not capable of detecting metals at the extremely low concentrations and in the presence of salt matrix interferences characteristic of sea water. Much of the matrix can be removed and the metals enriched by chemical separation. Tested ways of separating metals from sea water are precipitation, volatilization, solvent extraction, ion exchange, and electrodeposition (Table II).

Precipitation or crystallization is a classical method of separation. A prepared reagent can be added to a sample to collect the metals of interest. Generally, recoveries are good, but separations tend to be nonspecific and time consuming.

Volatilization has wide applicability in analytical chemistry. The volatile separations of arsenic, selenium, and mercury from their parent matrix are some common applications. Evaporation of sea water to concentrate metals is for all practical means useless because of the large amount of salt residue obtained.

Solvent extraction has been widely used to separate a single metal or a group of metals from water. Two popular metal extractants are dithizone chelation in chloroform and ammonium pyrolidine dicarbamate chelation (APDC) in methylisobutyl ketone (MIBK). Solvent extraction is relatively simple and rapid, but seldom does complete extraction of metals occur.

Electrolysis has been used to separate metal ions from sea water by deposition onto an electrode. Deposition behavior is influenced by many variables and must be closely controlled. The anlytical method of anodic stripping polarography is an application of the technique.

TABLE II

TECHNIQUES FOR CHEMICAL SEPARATION

Precipitation

Volatilization

Solvent Extraction

Ion Exchange

Electrodeposition

4. ANALYTICAL APPROACH

Refined instrumentation has proliferated the methods for trace metal analysis (Table III).

Atomic absorption is widely used for metals analysis. The instruments are durable and will detect some 40 elements. Detection is quite specific. Sensitivity is frequently near or lower than 1 ppm. Flameless atomic absorption is even more sensitive, but high background attenuation caused by sea water makes a precise direct analysis difficult.

Gas chromatography has been used to analyze metals as specific organocomplexes. The technique should have parts per billion detection ranges with an electroncapture detector. Sample preparation would require solvent extraction after formation of specific metal-chelates.

Anodic stripping voltammetry is an analytical application of electrochemistry. Metallic concentrations in water as low as 10^{-10} molar are detectable with 100 ml samples. Measurements must be carefully controlled to be reproducible and accurate. Anodic stripping voltammetry has been applied to shipboard analyses of Cd, Cu, Pb, and Zn. The instrument responds to free metal ions or complexes that break down during analysis. Selective ion probes may eventually provide a continuous monitoring system for heavy metals. Ion electrodes are available for detecting Cu, Pb, Ag, and Cd, but their application to sea water is limited.

Metals have long been determined by emission spectroscopy. It is very specific and can assay a sample for amny elements, simultaneously. Preliminary metal separation from sea water is required to attain detectable amounts and to reduce serious matrix interferences.

Spark source mass spectrometry could furnish a rapid and sensitive method of metals analysis, if matrix interferences from sea water are overcome. Costs for instruments, maintenance, and personnel make this a very expensive method.

Neutron activation analysis has been applied to sea water. Detection limits are within the range of a normal metals content of sea water. Multielement determinations can be done. Interferences from radioactive sodium would require chemical separation or long storage periods. The

TABLE III

PHYSICAL TECHNIQUES FOR MTALS ANALYSIS

Atomic Absorption Spectroscopy
Chromatography
Electrochemistry
Emission Spectroscopy
Mass Spectrometry
Radiochemistry
Spectrophotometry
X-Ray Spectroscopy

equipment is very expensive. The analysis techniques require highly trained laboratory personnel.

Spectrophotometry methods are capable fo providing reliable analyses at the ppm and below level for many metals. Spectrophotometers are durable and inexpensive instruments. Many physical and chemical factors influence precision and accuracy.

Multispectrometer X-ray fluorescence instruments are available that will detect up to 20 elements in a solid sample, simultaneously. Solid state detectors have improved detection limits, but not enough for the low metals level in dried sea water salts. Sea water sediments could be rapidly analyzed by X-ray fluorescence.

5. APPLICATIONS

Arsenic

The arsenic level in sea water has been reported to be 3 µg/l (30). A popular method of arsenic analysis has been by atomic absorption (13) with a closed system arsine generator. The detection limit of 5 µg/l can be lowered slightly by concentrating the sample before analysis. This can be done by water loss during a heated-digestion step. Differential pulse polarography has been used as an analytical technique for arsenic (15). Other inorganic ions may interfere, but the method is rapid and will detect arsenic in the µg/l range. Portmann and Riley (19) have described a way to separate and concentrate arsenic from sea water by co-crystallization with thionalide. The concentrate was reacted to form a molybdate blue complex for spectrophotometric measurement. They reported an arsenic recovery of 98%. The analysis required three days to complete.

Beryllium

The amount of beryllium in the open sea has been reported to be about 0.0002 µg/l (14). Ion exchange was used to concentrate the beryllium to a level that could be detected by emission spectroscopy. The beryllium detection limit by conventional atomic absorption is about 4 orders of magnitude higher than that normally found in the sea. A very large sample would be needed to obtain a detectable concentrate. Solvent extraction of chelated beryllium followed by detection with gas chromography has possible applications for sea water analysis. Ross and Sievers (25) have established a detection limit of 4×10^{-14}g Be with electron-capture detection of trifluoroacetylacetone chelated beryllium.

Cadmium

The quantity of cadmium usually present in sea water is near 0.1 µg/1 (23). Both neutron activation analysis and anodic stripping voltammetry have sufficient sensitivity for direct measurements at such a low concentration. Anodic stripping voltammetry is more easily available and suitable for water chemists. A method using anodic stripping voltammetry for the determination of Cd, Cu, Pb, and Zn in sea water has been reported by Rojahn (24). His technique was rapid, sensitive, and the reproducibility was very good. Atomic absorption is an often used way to determine cadmium in high salt systems after ion exchange concentration and separation from the parent matrix. Although ion exchange separation is time consuming, it is simple and with care, precision can be within 5%. The method for cadmium analysis used in our laboratory is similar to that reported by Windom and Smith (32), except we use Dowex A-1 resin. A more rapid determination of cadmium at higher levels can be made by solvent extraction (10).

Chromium

The level of chromium in typical sea water is about 0.4 µg/l (8). Concentration by one order of magnitude will place the chromium level within the detection limit of conventional atomic absorption. Solvent extraction by APDC/MIBK will accomplish the task after Cr^{3+} is oxidized because only Cr^{6+} chelates with the APDC (16). The concentrate is easily within the detection range of analysis by atomic absorption equipped with a flameless graphite furnace device (18). Chau, Sim, and Wong (7) used a technique that lowered the analytical range for chromium by atomic absorption to 0.2 µg/1 with a reproducibility of ±0.06 µg/l. They extracted chromium acetylacetonate into MIBK after co-precipitation of chromium with Fe (OH) from liter samples of sea water.

Cobalt

The cobalt content of sea water is about 0.1 µg/1 (30). Cobalt has been satisfactorily determined by atomic absorption after separation and concentration from sea water by either ion-exchange (32) or solvent extraction (12, 18). Cobalt as low as 0.001 µg/l can normally by measured in neutron activated sea salts after a two-month storage period.

Copper

The copper content of sea water is about 1 µg/l (30). Copper has been analyzed by atomic absorption after concentration by solvent extraction (28) or ion exchange (32). Riley and Taylor (21) report that copper can be retained quantitatively from filtered sea water by Chelex-100 and eluted completely with 2N nitric acid. Kuwata, Hisatomi, and Haseqawa (10) successfully extracted copper from sea water with sodium diethyldithiocarbamate into MIBK. Rojahn (24) has demonstrated that anodic stripping voltammetry works well for direct analysis of copper in sea water.

Iron

The amount of iron normally present in sea water is near 10 µg/l (30). Numerous colorimetric methods are available for determination of soluble iron in sea water. The phenanthroline color is commonly used. A procedure has been described by Strickland and Parsons (29). The method is practically free of interferences from normal sea water constituents. Segar and Gonzales (26) have demonstrated that iron can be determined directly by atomic absorption with a flameless graphite furnace device. Iron can also be analyzed by flame atomic absorption after concentration by solvent extraction (28).

Lead

Lead in sea water is normally found at a concentration of 0.03 µg/l (30). A good anodic stripping voltammetry technique for shipboard analysis for lead has been described by Zirino and Healy (33). Rojahn (24) reported good reproducibility for lead analysis in sea water by a similar polarographic technique. Lead can be concentrated from sea water for analysis by atomic absorption using ion exchange chelation (21). A rather large sample would be needed to obtain sufficient lead for flame atomic absorption analysis.

Manganese

The manganese concentration in sea water is near 2 µg/l (30). An atomic absorption analysis method with manganese concentration by ion exchange has been reported by Riley and Taylor (23). Their accuracy and precision was quite good. A very sensitive malachite green spectrophotometric method for direct manganese analysis has been outlined by Strickland and Parsons (29).

Mercury

The normal mercury content of sea water is 0.03 µg/l (30). A simple technique of concentrating mercury from two to 10 liters sea water into 20 ml of scrubbing solution has been described by Topping and Pirie (31). Their technique permits analysis by flameless atomic absorption of samples containing as low as 0.002 µgHg/l. A dithizone-chloroform extraction technique similar to that described by Chau and Saitoh (6) is used in our laboratory. Mercury levels in samples as low as 0.05 µg/l have been detected using a conventional flameless atomic absorption unit. Several commercial mercury vapor analyzers are available that have detection limits below that normally found in sea water. Chemical separation of mercury from a sea water aliquot can be achieved by selective deposition onto cadmium sulfide pads or glass disks impregnated with gold. The collectors can be heated to volatilize mercury for measurement. Anderson, Evans, Murphy, and White (1) could detect as low as 0.001 µg Hg by this technique. A sensitive direct measurement device has been described by Braman (2). His detector was a rubber membrane probe permeable to reduced mercury and helium carrier gas. The gas stream passes through a dc discharge cell from which the mercury emission intensity is recorded. Water samples containing from 0.01

to 0.16 µg/1 were analyzed without any apparent matrix interferences.

Molybdenum

The molybdenum content of sea water is about 10 µg/l (30). A technique for ion exchange concentration has been described by Riley and Taylor (22). The molybdenum concentrate was measured spectrophotometrically. Molybdenum has been separated from sea water with the chelates chitosan and diethylaminoethylcellulose by Muzzarelli and Rocchetti (17). They analyzed the concentrate by flameless atomic absorption. A molybdenum collector of thorium hydroxide was used by Kim and Zeitlin (9). Final determination was done spectrophotometrically with the Mo-thiocyanate complex. They reported a molybdenum recovery near 100%.

Nickel

The nickel content of sea water is about 2 µg/l (30). Many of the chemical separation techniques of ion exchange (21) and solvent extraction (3) for cadmium are also applicable to nickel. Rampon and Cuvelier (20) reported that nickel in sea water can be determined at 1 µg/l levels by atomic absorption after extraction with dimethylgloximate in chloroform.

Selenium

The selenium level in sea water is about 0.4 µg/l (30). Selenium, like arsenic, can be determined by atomic absorption with the closed system generator. The sample level need be at least 2 µg/l. A spectrophotometric method using diaminobenzidine has been reported by Chau and Riley (5) for selenium analysis of sea water. They concentrated selenium by co-precipitation with iron hydroxide. A very sensitive and rapid technique has been devised by Shimoishi (27) using an electron-capture detector on a gas chromatograph. As low as 0.02 µg/l selenium was detected.

Silver

The silver concentration in sea water is about 0.05 µg/l (30). The very high sensitivity for silver by flameless atomic absorption makes it an attractive choice for analysis. Solvent extraction of Ag-dithizonate could probably be used to separate silver from sea water. A spectrophotometric method for silver after co-crystallization with thionalide has been described by Lai and Weiss (11). The crystal concentrates were digested in acid. Estimation of silver was made from the red-violet color reaction with rhodanine.

Vanadium

The vanadium content of sea water is about 2 µg/l (30). Spectrophotometric determination of vanadium with diaminobenzidine after concentration from sea water by ion exchange has been described by Riley and Taylor (22). They obtained a complete recovery of added vanadium using Chelex-100 resin. Although the precision was relatively poor, Segar and Gonzalez (26) have shown that direct analysis by flameless atomic absorption can be achieved.

Zinc

Normal sea water contains about 10 µg/l of zinc (30). Techniques of anodic stripping polarography and atomic absorption spectroscopy have been used successfully in numerous laboratories to measure zinc in sea water. Burrell and Wood (4) used a tantalum sample boat accessory with atomic absorption to directly determine zinc in 0.25 ml sea water samples. They reported a detection limit of 0.0002 µg Zn. A zinc extraction procedure by Nix and Goodwin (18) for lake water should be applicable to sea water. Chemical separation of zinc by Chelex-100 resin from sea water has been satisfactorily done by Windom and Smith (32). Atomic absorption was their choice for the final analysis. Zirino and Healy (33) described an anodic stripping voltammetry method used for shipboard analysis of zinc. They provided evidence that pH dependent metal complexes occur in sea water. Their lowest detectable level of zinc was 0.2 µg/l.

Table IV lists the concentration of metals often found in sea water and the methods that have been successfully used for the analysis of the particular metals.

TABLE IV

APPLICATIONS

Element	Normal Amount In Seawater µg/l	Method	Reference
Arsenic	3.	Atomic Absorption	Manning (1971)
		Differential Pulse Polarography	Myers & Osteryoung (1973)
		Co-crystallization - Spectrophotometry	Portmann & Riley (1964)
Beryllium	0.0002	Ion Exchange - Emission Spectroscopy	Merrill, et. al. (1960)
		Gas Chromography	Ross & Sievers (1972)
Cadmium	0.1	Anodic Stripping Polarography	Rojahn (1972)
		Ion Exchange - Atomic Absorption	Windom & Smith (1972)
Chromium	0.4	Solvent Extraction - Atomic Absorption	Chau, et. al. (1968)
		Flameless Atomic Absorption	Nix & Goodwin (1970)
Cobalt	0.1	Ion Exchange - Atomic Absorption	Windom & Smith (1972)
		Solvent Extraction - Atomic Absorption	Lee & Burrell (1972)
Copper	1.	Ion Exchange - Atomic Absorption	Riley & Taylor (1968)
		Solvent Extraction - Atomic Absorption	Kuwata, et. al. (1971)
		Anodic Stripping Polarography	Rojahn (1972)
Iron	10.	Flameless Atomic Absorption	Segar & Gonzalez (1972)
		Spectrophotometry	Strickland & Parsons 1968
		Solvent Extraction - Atomic Absorption	Spencer & Brewer (1969)
Lead	0.03	Anodic Stripping Polarography	Zirino & Healy (1972)
		Ion Exchange - Atomic Absorption	Riley & Taylor (1968)
Manganese	2.	Ion Exchange - Atomic Absorption	Riley & Taylor (1968)

Element	Normal Amount In Seawater µg/l	Method	Reference
Mercury	0.03	Flameless Atomic Absorption Cold Vapor Atomic Absorption Solvent Extraction - Cold Vapor AA Membrane Probe	Anderson, et. al. (1971) Topping & Pirie (1972) Chau & Saitoh (1970) Brauman (1971)
Molybdenum	10.	Coprecipitation - Spectrophotometry Ion Exchange - Spectrophotometry	Kim & Zeitlin (1970) Riley & Taylor (1968)
Nickel	2.	Solvent Extraction - Atomic Absorption Solvent Extraction - Atomic Absorption	Brooks, et. al. (1967) Rampon & Cuvelier (1972)
Selenium	0.4	Coprecipitation - Spectrophotometry Gas Chromatography	Chau & Riley (1965) Shimoishi (1973)
Silver	0.05	Cocrystallization - Spectrophotometry	Lai & Weiss (1962)
Vanadium	2.	Ion Exchange - Spectrophotometry Flameless Atomic Absorption	Riley & Taylor (1968) Segar & Gonzalez (1972)
Zinc	10.	Flameless Atomic Absorption Ion Exchange - Atomic Absorption Anodic Stripping Polarography	Burrell & Wood (1969) Windom & Smith (1972) Zirino & Healy (1972)

6. SUMMARY

Analytical methods are described to determine As, Be, Cd, Cr, Co, Cu, Fe, Pb, Mn, Hg, Mo, Ni, Se, Ag, V, and Zn in sea water.

Analysis of all metals can be accomplished with various degrees of convenience by atomic absorption. Direct analysis by flameless atomic absorption has many desirable attributes, but utilization cannot be maximized until the interferring action of sea water components is overcome. Anodic stripping voltammetry is becoming increasingly useful for sea water analysis. Direct measurements for Cd, Cu, Pb, and Zn have been made. Major ions in sea water do not interfere. Neutron activation analysis and spark source mass spectrometry have been utilized as analytical techniques. Gas chromatography has possible applications for sea water metal analysis.

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RECENT STUDIES ON BIOLOGICAL EFFECTS OF CRUDE OILS AND OIL-DISPERSANT MIXTURES TO RED SEA MACROFAUNA

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INTRODUCTION

With the closure of the Suez Canal in 1967, the deep-water harbor at Elat, Israel, became increasingly important as an oil terminal (Figure 1). In the 12 months ending in July 1973, more than 25 million tons of crude oil were unloaded, most of which was transported by supertankers in the 200,000-250,000 ton class. From Elat, oil is transported via the Elat-Ashkelon pipeline and is eventually processed at European or Israeli refineries. Spills are common at Elat; a majority of them were estimated at less than 1,000 liters, but at least two greater than 10,000 liters were observed within the past year. Oil spill countermeasures are extensive and include mechanical containment devices around vessels unloading, use of straw and other oil-absorbing materials in the event of spillage, cessation of unloading operations with the advent of southerly winds in order to protect the luxury bathing beaches to the north, and heavy financial penalties to polluters. The use of chemical oil dispersants are prohibited at Elat in order to minimize damage to the biota; however, this is not rigidly enforced.

Because virtually nothing was known regarding the influence of crude oil or chemical oil counteractants on local coral reef ecosystems, the Hebrew University in 1972 initiated a continuing series of investigations in this subject area. This account summarizes progress during the first year of laboratory studies and is extracted wholly from the findings of Cohen (4), Eisler (5, 6, 7), and Eisler and Kissil (8). Specifically, we report on acute toxicity to representative species of marine macrofauna of two common grades of crude oils (which together comprise more than 80% of all crude oil unloaded at Elat), a chemical oil

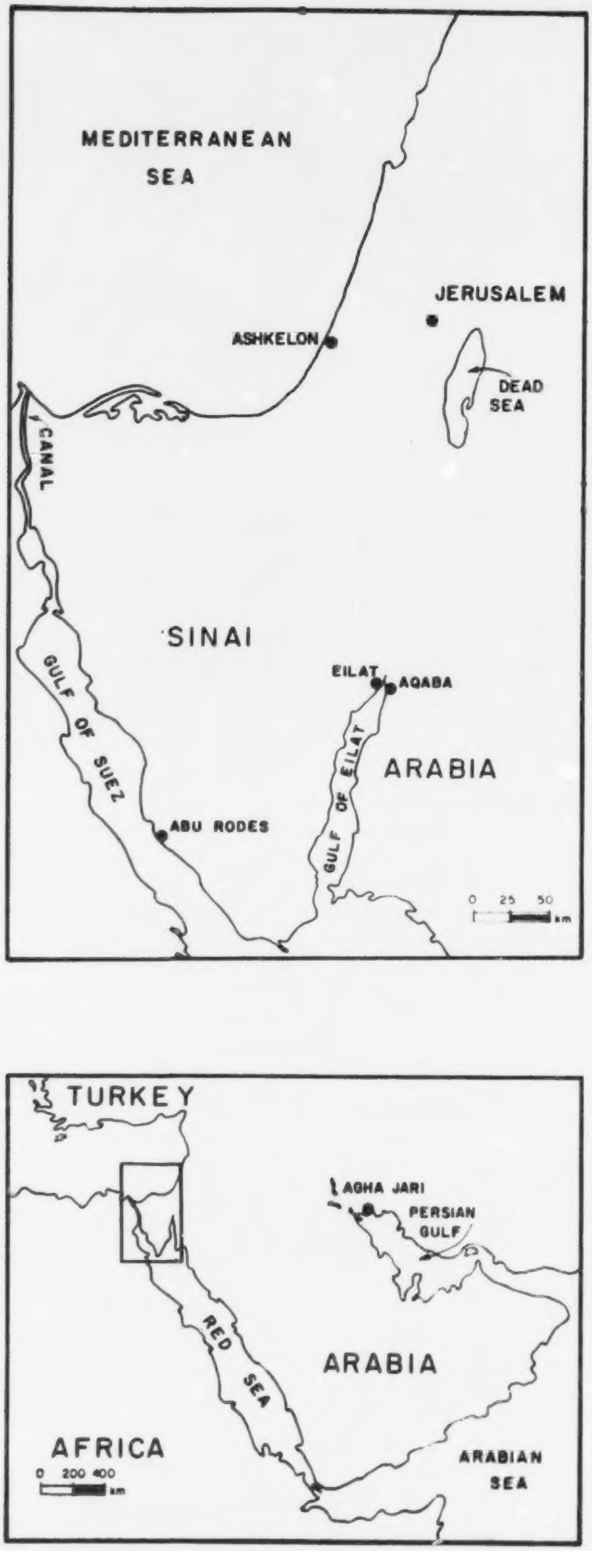
MEDITERRANEAN SEA
JERUSALEM
ASHKELON
DEAD SEA
CANAL
SINAI
EILAT
AQABA
GULF OF SUEZ
GULF OF EILAT
ARABIA
ABU RODES
0 25 50 km
TURKEY
AGHA JARI
PERSIAN GULF
RED SEA
ARABIA
AFRICA
0 200 400 km
ARABIAN SEA

Figure 1. Geographic Loaction of Eilat

dispersant used extensively in Northern Israel, and mixtures of oil and dispersant at realistic application levels. Bioassays were conducted under static as well as continuous flow conditions. Depth-toxicity interactions were evaluated using deep (2.0 m) tanks. Sublethal and latent effects of crudes and oil-dispersant mixtures on physiology, metabolism, and behavior were investigated as were short-term degradation and bioaccumulation of oil.

TEST TOXICANTS

Three materials were evaluated: Iranian crude oil from the Agha-Jari field located at the head of the Persian Gulf (Figure 1), Sinai crude oil from the vicinity of Abu Rodes situated on the Gulf of Suez (Figure 1), and a chemical oil dispersant used extensively on the Mediterranean Coast of Israel. Oil samples were kindly provided by the Elat-Ashkelon Pipeline Company and were taken from tankers unloading at Elat in September, 1972. These were stored in tightly sealed 25 liter containers until use. Iranian crude, which comprises about 60% of the total unloaded at Elat, when compared to Sinai crude (20% of total) is low is sulfur, asphaltenes, carbon residues, and especially viscosity (Table 1). The Sinai crude transported by tankers is a mixture of 20% offshore field and 80% onshore field (Table 1). Dispersant samples were supplied by Chemotas Ltd. of Tel Aviv. Chemotas stated that the dispersant consisted of 15% surfactant and 85% solvent. Composition of the surfactant fraction, according to Chemotas spokesman, is: 70% ethylene oxide; 15% linear alcohols in the C11-C15 range; 10% organic impurities, mostly amines; and 5% water. The solvent fraction consits of about 65% aromatics and 35% high boiling aliphatics.

ACUTE TOXITY

Static Jar Tests

Methods: Static bioassays were conducted in wide mouth glass jars containing 3 liters of aerated, unfiltered seawater. Each test was conducted at a salinity of 41 o/oo, a temperature of 22±1°C, dissolved oxygen greater than 4.0 mg/l, and pH of 8.1±0.1 unit; these conditions approximated those of nearby waters as described by Oren (11). The test animals were adults from locally abundant groups of coelenterates (1 species), molluscs (4 species), crustaceans (2 species), echinoderms (1 species), and juvenile teleosts (2 species). Species used were: Heteroxenia fuscescens, an

	Field Location		
Properties	Agha Jari field, Persian Gulf, offshore. (1)	Belayim field, Siani, onshore (2)	Belayim field, Sinai, offshore (2)
Spec. grav. at 60°F.	0.847	0.922	0.875
API	35.6	21.8	30.2
Kinematic viscosity			
at 50°F	14.4	796.0	134.0
at 100°F	5.66	93.0	13.0
Pour point	+10	+6	+6
Sulfur, wt %	1.35	3.29	1.8
Asphaltenes, wt %	0.60	7.7	1.54
Carbon residue, wt%	3.7	10.2	4.8

(1) from Fallah et al (1972)
(2) data supplied by Israel Institute of Petroleum. Sample tested for toxicity was a mixture of 80% onshore field, 20% offshore.

Table 1. Some properties of crude oils used in studies on toxicity of oils to Red Sea macrofauna.

octocoral; *Drupa granulata*, a gastropod drill: *Nerita forskali*, a littoral gastropod; *Mytilus variabilis*, a mussel; *Acanthopleura haddoni*, a chiton; *Calcinus latens*, a hermit crab; *Palaemon pacifica*, a shrimp; *Echinometra mathei*, a sea urchin; *Parupeneus barberinus*, goatfish; and *Siganus rivulatus*, rabbitfish.

Assay organisms were added to the jars about 30 minutes before the test substances. Agitation of the media-toxicant solution as recommend by LaRoche, *et al* (10) was not essential according to results of preliminary studies that we conducted. Details of methodology and procedures are listed in LaRoche, *et al* (10) as modified by Eisler (5).

Results: Concentrations of all compounds tested that were fatal to 50% of the individuals in 168 hours (LC-50 (168 hr.) are presented in Table 2. From these data we concluded that both grades of crude oil are relatively innocuous when compared to the dispersant; that toxicity of oil-dispersant mixtures reflect the biocidal properties of the dispersant alone; and that fishes and crustaceans are among the most sensitive groups tested. Similar results were reported by Carthy and Arthur (3) and LaRoche, *et al* (10).

In another series of studies with juvenile rabbitfish, we investigated the effect of time in the aerated assay medium on toxicity in high concentrations of Iranian crude oil, Sinai crude oil, and dispersant. For the concentrations tested each compound behaved differently during a maximum period of 168 hours in the assay medium before living organisms were added. For example, Iranian crude oil became progressively less toxic with time, and the dispersant lost all of its biocidal properties within 2 hours (Table 3). But Sinai crude became increasingly more toxic with time (Table 3). To account for these phenomena it would appear that the more volatile fractions of Iranian crude, as well as dispersant, must contain most of the acutely lethal components; for Sinai crude, the non-volatile components would be the most toxic. Additional bioassays with rabbitfish confirmed that the surfactant fraction of the dispersant, *i.e.* the ethylene oxide-containing fraction, contained virtually all of the toxic properties. Also, the lower boiling components of Iranian crude and the highest boiling fractions of Sinai crude were indeed the most toxic (Table 4).

Animal	Iranian crude oil	Sinai crude oil	Dispersant	Oil-dispersant mixtures 1/
Coelenterates				
octocoral 2/	12	30+	.018	.110+
Molluscs				
drill	30+	30+	.064	-----
snail	17	30+	.045	.152
mussel	30+	30+	.018	-----
chiton	30+	30+	.013	-----
Crustaceans				
hermit crab	21	30+	.006	-----
shrimp	2	16	.006	.096
Echinoderms				
urchin	30+	30+	.006	.061
Teleosts				
goatfish 3/	6	16	.006	-----
rabbitfish	0.8	15	.010	.150

1/ Values are in ml/l test compound added to jars at start.

2/ 96 hour values

3/ 48 hour values

Table 2. Concentrations of two crude oils, a chemical dispersant, and oil-dispersant mixture (10 parts oil - 1 part dispersant v/v) fatal to 50% of selected species of Red Sea macrofauna in 168 hours.

Fish added to medium - hours after test substance	Iranian crude (1.9 ml/1)	Sinai crude (22 ml/1)	Dispersant (0.020 ml/1)
168	12	75	0
120	18	75	0
72	44	63	0
24	63	50	0
2	—	—	0
1	—	—	25
0	75	25	75
Controls	0	0	0

Table 3. Effect of time in aerated medium on toxicity of high concentrations of two crude oils and one dispersant to juvenile rabbitfish. Values represent percent dead at 168 hours (n = 6 to 8 fish/time interval).

Boiling fraction	Type oil	% dead in 96 h
Low, <145°C	Iranian	100
Intermediate, 145-210°C	Iranian	50
High, >210°C	Iranian	0
Controls	-------	0
Low, <80°C	Sinai	0
Intermediate, 80-115°C	Sinai	0
High, >115°C	Sinai	50
Controls	-----	0

Table 4. Toxicity of three boiling fractions of two crude oils to juvenile rabbitfish. Values equal percent dead at 96 hours during exposure to 3.3 ml/l under static conditions.

Continuous Flow Tank Tests

Methods: Assay tanks (Figure 2) were 2.0 meters high, 1.0 meter in diameter, with a window 60 cm wide by 125 cm high. Each tank was divided into two compartments by perforated partitions placed 0.2, 1.0, and 1.8 m from the surface. Construction of tank and partitions was fiberglass. Unfiltered seawater was introduced in a spray pattern via perforated pipes at the rate of about 5 liters/minute from a constant head tank. This flow is equivalent to a water turnover rate of about 4.8 daily. All incoming water passed through the oil film; but data on degree of mixing and turbulence are lacking. Waste water exited via standpipes situated below the bottom partition then traversed oil traps and sand filters before discharge into the Gulf of Elat. Seawater composition was as described in the previous section. A more detailed account of methods and procedures is presented in Eisler and Kissil (8) and Eisler (7).

Assay animals were introduced 1-2 days prior to the toxicants. Because of space limitations only a limited number of comparatively high concentrations were tested. The highest level of oils added to each tank was 10 ml/l; this is equivalent to 15 liters added at the surface. In terms of liters per m^2 surface area, a concentration of 10 ml/l for the tank tests equaled 19.10; for the jar tests this was 1.33.

Results: A comparison of acute toxicity values to Red Sea macrofauna derived from static jar tests and from continuous flow tank tests is shown in Table 5. In the 20 instances of paired observations for which comparisons are possible, toxicity values derived from tank tests were higher, i.e. test compounds were less toxic in 11 of the 20 cases and lower in only one instance (crustaceans vs dispersants); in the remaining 8 cases there was insufficient data to reach a conclusion (Table 5). It is probable that the lower mortality observed in tanks when compared to jars is associated with lower biomass/l medium as well as metabolite and toxicant removal by the constant flushing action.

For all species and all compounds assayed in the large tanks, survival was the same in both compartments in 40 out of 55 tests (Table 6). In the 15 instances where differential survival was observed between compartments, it was the lower compartment (1.0 to 1.8 m from surface) that exhibited higher survival in 14 out of 15 instances (Table 6). It appears that a depth protective effect exists and

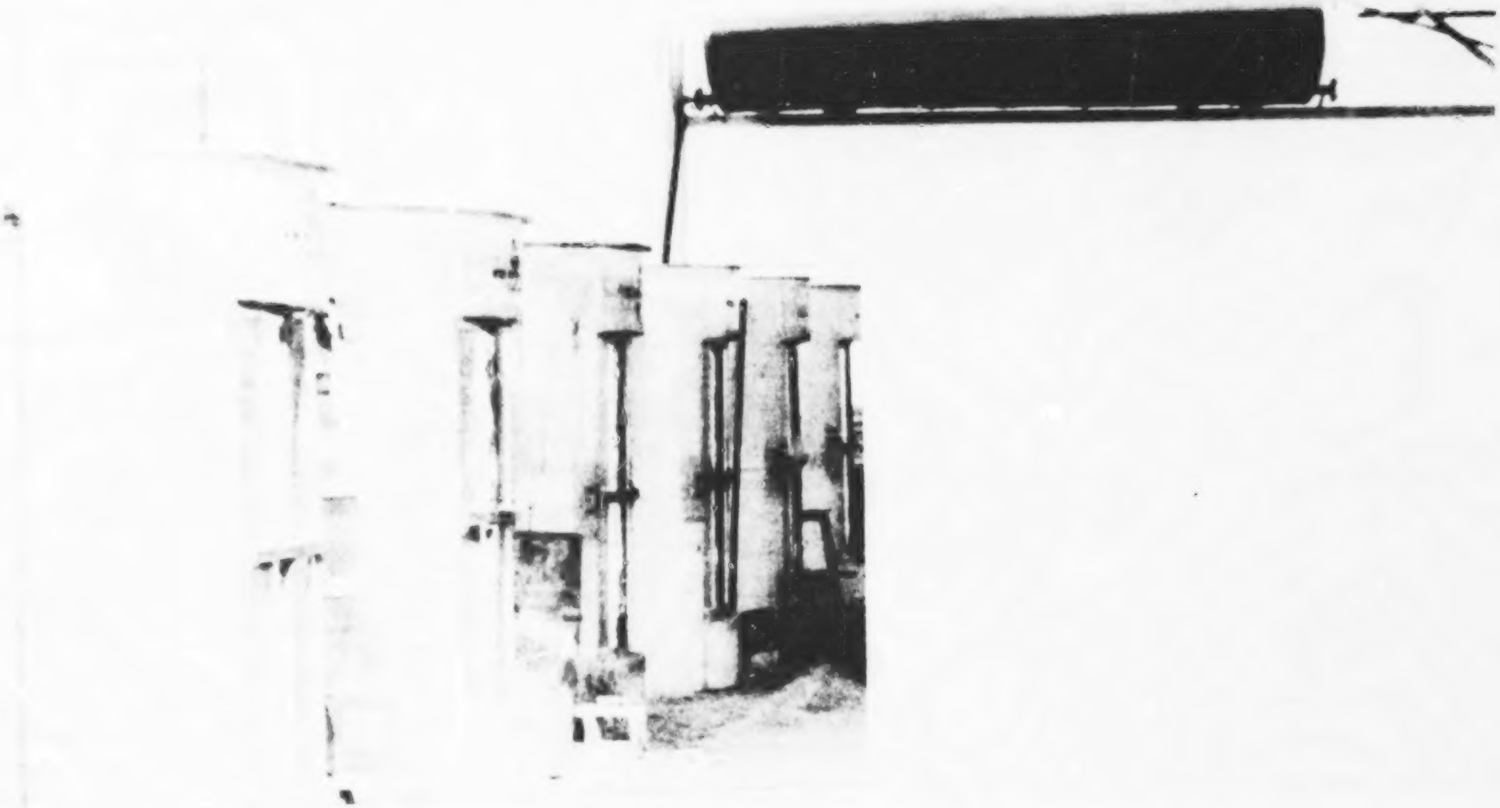

Fig. 2 Constant head and continuous flow tanks system used in oil and dispersant toxicity bioassays.

	Iranian Crude Oil		Sinai Crude Oil		Chemical Dispersant		Oil-Dispersant Mixtures[6/]	
	Jar	Tank	Jar	Tank	Jar	Tank	Jar	Tank
Coelenterates[1/]	1	10+	30+	10+	0.010	0.030	0.110+	0.110+
Molluscs[2/]	3 to 10	10+	10 to 30	10+	0.003 to 0.020	0.010 to 0.100	0.110	0.110+
Crustaceans[3/]	1 to 3	10+	3 to 10	10+	0.003	0.001	0.011 to 0.033	0.110
Echinoderms[4/]	30+	10+	30+	10+	0.003	0.003	0.033	0.110
Teleosts[5/]	0.3 to 1	10+	3 to 10	10+	0.003	0.010	0.033 to 0.110	0.110+

1) Organism assayed for both jar and tank tests was *Heteroxenia fuscescens*, an octocoral.
2) For jar tests: *Drupa granulata* (gastropod), *Nerita forskali* (gastropod), *Mytilus variabilus* (mussel), *Acanthopleura haddoni* (chiton): tank tests: *Pinctada fucada* (pearl oyster), *Trochus dentatus* (gastropod), *Circe crocea* (clam).
3) Jar tests: *Palaemon pacifica* (shrimp), *Calcinus latens* (hermit crab); tank tests: *Panulirus pencillatus* (lobster).
4) Jar tests: *Echinometra mathei* (short-spined sea urchin); tank tests: *Diadema setosum* (long-spined sea urchin).
5) Jar tests: *Siganus rivulatus* (rabbitfish), *Parupeneus barberinus* (goatfish); tank test; *S. rivulatus*.
6) Mixtures contain 91% oil, 9% dispersant v/v.

Table 5. Comparison of crude oil and oil-dispersant toxicity values to Red Sea fauna derived from static jar tests and from continuous flow tank tests. Values are in ml/l toxicant added to medium at start allowing at least 90% survival in 168 hours.

	Total number tests	No. tests with differential survival between upper and lower compartments	No. tests with survival higher in	
			upper (0.2-1.0 m) compartment	lower (1.0-1.8 m) compartment
Dispersant	19	8	0	8
Iranian crude oil	8	2	0	2
Sinai crude oil	7	1	0	1
Oil-dispersant mixtures	14	4	1	3
Controls	7	0	0	0
Total	55	15	1	14

Table 6. Tank tests: Effect of distance from surface on survival of selected species of Red Sea macrofauna. Exposure was for 168 hours to various concentrations of chemical dispersant, crude oils, and oil-dispersant mixtures.

that this observation merits further elucidation in future toxicity testing of oils and oil counteractants.

SUBLETHAL EFFECTS

Iranian Crude

After immersion for 168 hours under static assay conditions, several species exhibited abnormal behavior patterns when compared to controls. Mussels, for example, did not adhere to the walls of the test jar or to other mussels by byssal thread attachment, were slow to close valves in response to mechanical stimuli, and secreted copious quantities of mucous. About 60% of the mussels immersed in 30 ml/l were afflicted, 40% at 3 ml/l, 10% in 1 ml/l and none at lower concentrations tested. Snail (*Nerita*) and drill (*Drupa*) distress syndromes were characterized by extreme sluggishness in closure of operculum following tactile stimulation, inability to complete closure of operculum, failure to adhere to sides or bottoms of jar, and excessive mucous secretion. For snails, 80% showed this pattern at 10 ml/l, 40% at 3 ml/l, and none at lower levels of Iranian crude. Drills were more resistant with only 10% in stress at 30 ml/l and none at lower levels. Rabbitfish exhibited a lowering in blood hematocrit values and a pronounced enlargement of the liver (Table 7); these results were statistically significant at the 0.01 level. However, all rabbitfish survived at least six months posttreatment in flowing seawater aquaria with apparently normal appetite and growth; unfortunately, data are lacking on posttreatment hematocrits and SLI values.

In flowing water tank tests, octocorals were affected during immersion in 10 ml/l Iranian crude to the extent of reduction in tentacular pulsation rate. Reduction was most pronounced (100% inhibition in 96 hr) among those held nearest (0.2 m) the surface and least pronounced (40% inhibition in 96 hr) among groups held furthest (1.8 m) from the surface; intermediate values were observed at intermediate depths.

Sinai Crude

Mussels and snails that survived immersion for 168 hours in high concentrations under static conditions showed distress, as described previously. More than 90% of mussels held in 30 ml/l were abnormal at 168 hr; this dropped to 60%

Treatment	Hematocrit	SLI
Iranian crude		
0.30 ml/1	-7	+55
1.00 ml/1	-26	+105
Sinai crude		
10.0 ml/1	-16	+158
Dispersant		
0.001 ml/1	-10	+2
0.003 ml/1	+2	+5
0.010 ml/1	-18	+21

[1] Control values were 47.18 for hematocrit and 1.155 for somatoliver index.

Table 7. Effect of two crude oils and a dispersant on blood hematocrit and somatoliver index (liver wt/body wt X 100) of juvenile rabbitfish, *Siganus rivulatus* surviving exposure for 168 hours. Values are percent deviation from controls.[1]

at 10 ml/l and zero at 3 ml/l and lower. An identical pattern was observed for snails.

Rabbitfish subjected to 10 ml/l Sinai crude in jars showed a lowering of blood hematocrits and marked increases in liver size (Table 7). As was true for Iranian crude, there were no deaths during a posttreatment observation period of six months.

Dispersant

In jar tests, stress was observed in 30% of mussels held at 0.02 ml/l, with all animals outwardly normal at lower concentrations. There is a trend towards lower blood hematocrits and increasing SLI among rabbitfish (Table 7), but these differences were not statistically significant at the 0.01 level.

Oil-Dispersant Mixtures

Snails exhibited distress after immersion in oil-dispersant mixtures for 168 hr. More than 80% appeared abnormal in a mixture of 0.1 ml/l Iranian crude plus 0.01 ml/l dispersant, the lowest concentration tested. At higher sublethal levels tested, 100% manifested the stress syndrome described earlier.

LATENT EFFECTS (MOLLUSCS)

This section summarizes effects of high sublethal concentrations of Iranian crude oil and dispersant on posttreatment predation rate of the gastropod, *Drupa granulata*, on the mussel, *Mytilus variabilis* and on number of egg cases deposited by *Drupa*.

Oil

Groups of mussels and drills were exposed separately for 168 hr to 10 ml/l of Iranian crude in static jar assays. Afterwards, mussels (n = 65) and drills (n = 15) were placed in flowing seawater aquaria until 95% or more of the mussels were consumed by the drills. Four combinations were possible using oil-exposed drills (D^+), oil-exposed mussels (M^+), control drills (D^-), and control mussels (M^-): namely, D^+M^+, D^+M^-, D^-M^+, and D^-M^- (controls). In 28 days almost all of the mussels in the D^-M^- group had been consumed compared to 1/3 this level in the D^+M^+ group, and 1/2 this level when only one species, either predator or prey, was similarly exposed (Figure 3). We speculate that at least two mechanisms may be operable to account for this phenomenon: (a) whereby oil taken in by mussels acted as a drill repellant; (b) another involving inhibition of the drill's radular apparatus or digestive enzymes; the effects are apparently cumulative. The effect of different time-concentration combinations other than 168 hr-10 ml/l oil on posttreatment predation rate is unknown and appears to require additional research.

The average number of egg cases deposited by individual *Drupa* over the 28 day period ranged between 4.2 and 14.2 (Figure 4). Fecundity of *Drupa* seems to be a direct function of mussel consumption (Figure 3); however, more research is needed to establish this pattern conclusively.

Dispersant

Immersion of *Drupa* and *Mytilus* in 0.003 ml/l for 168 hr did not affect posttreatment predation rate over a period of 32 days. But fecundity of untreated drills preying on untreated mussels was 7 times higher than groups where both had been initially subjected to dispersant and 3 to 5 times higher than groups where only one species had been subjected to dispersant. We concluded that high sublethal levels of the dispersant tested interferes with egg case deposition by *Drupa* during the first month posttreatment.

DEGRADATION

Gas chromatogams of crude oil from the Agha Jari field indicate that the most prominent peaks are those of normal paraffins, extending from at least decane ($C_{10}H_{22}$) to pentatriacontane ($C_{35}H_{22}$) with the majority in the C_{12}-C_{14} range. Details of the analytical procedure are presented by Cohen (1973). Gas chromatograms of weathered oil samples taken from the large tanks after 96 hr exposure at 23°C suggest the loss of low boiling compounds in the C_{10}-C_{15} range (Figure 5). This observation lends support to the findings of Blumer *et al* (2) that evaporation and dissolution are the initial degradation processes.

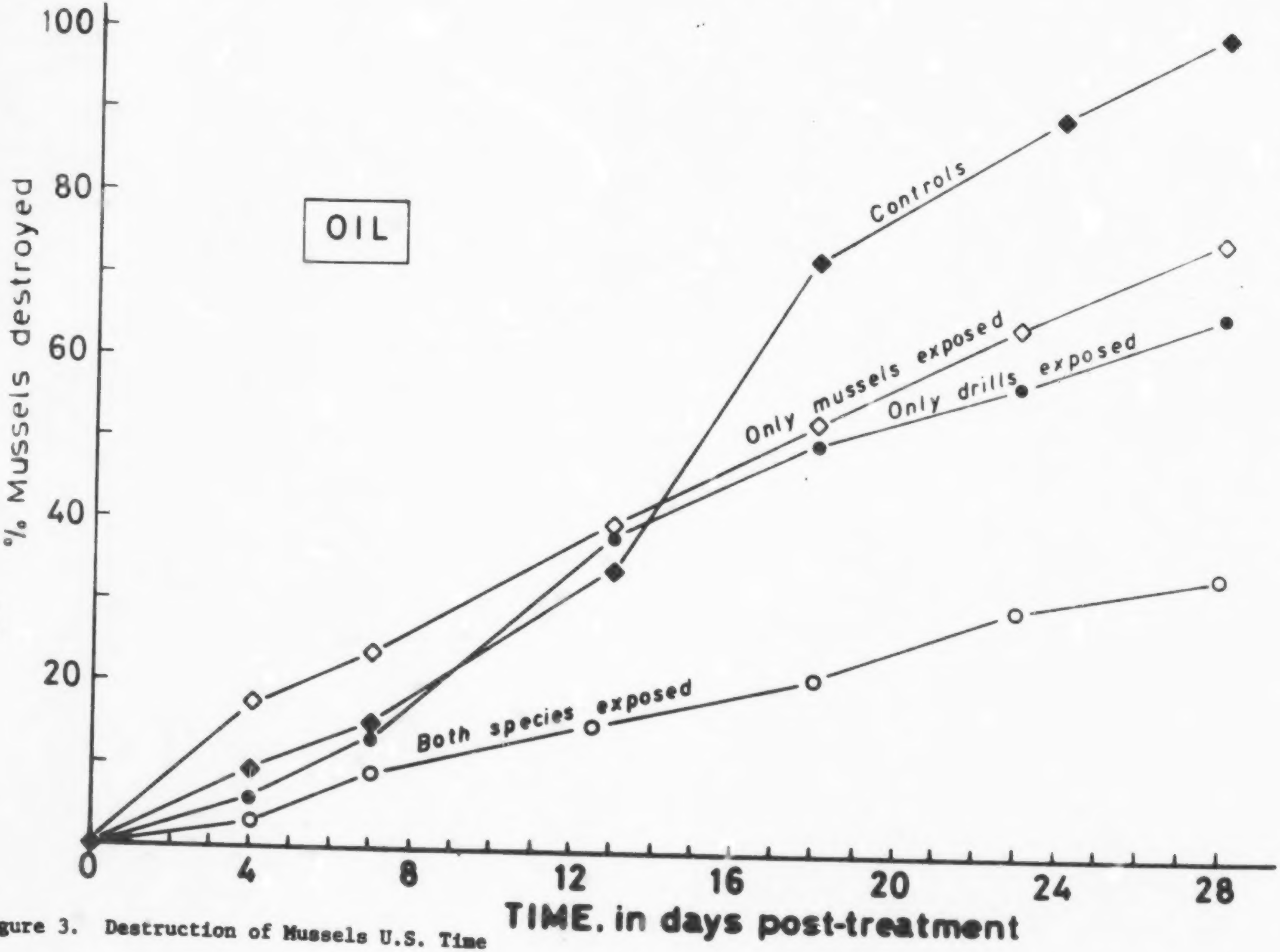

Figure 3. Destruction of Mussels U.S. Time

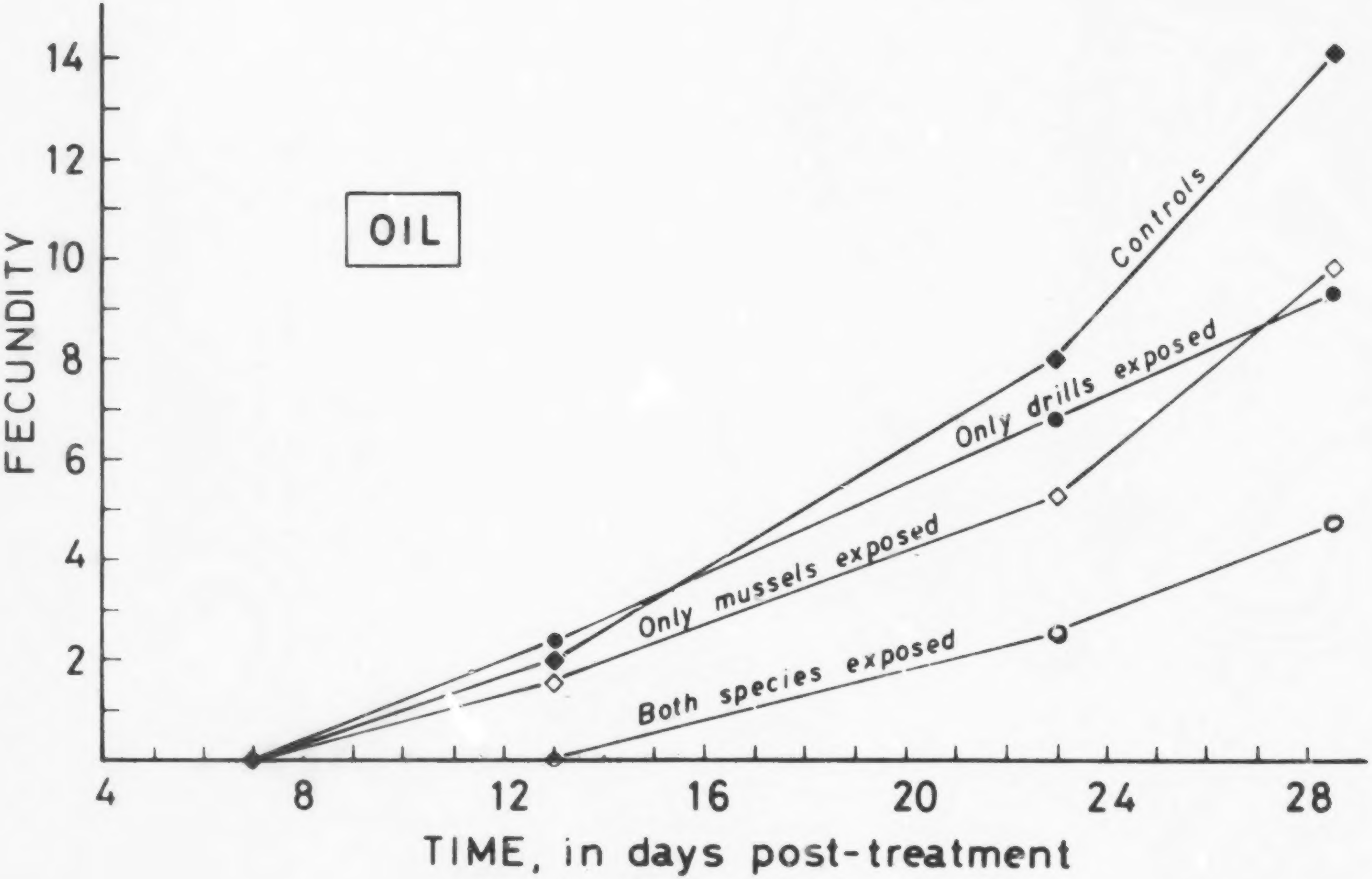

Figure 4. Deposition of Egg Cases U.S. Time

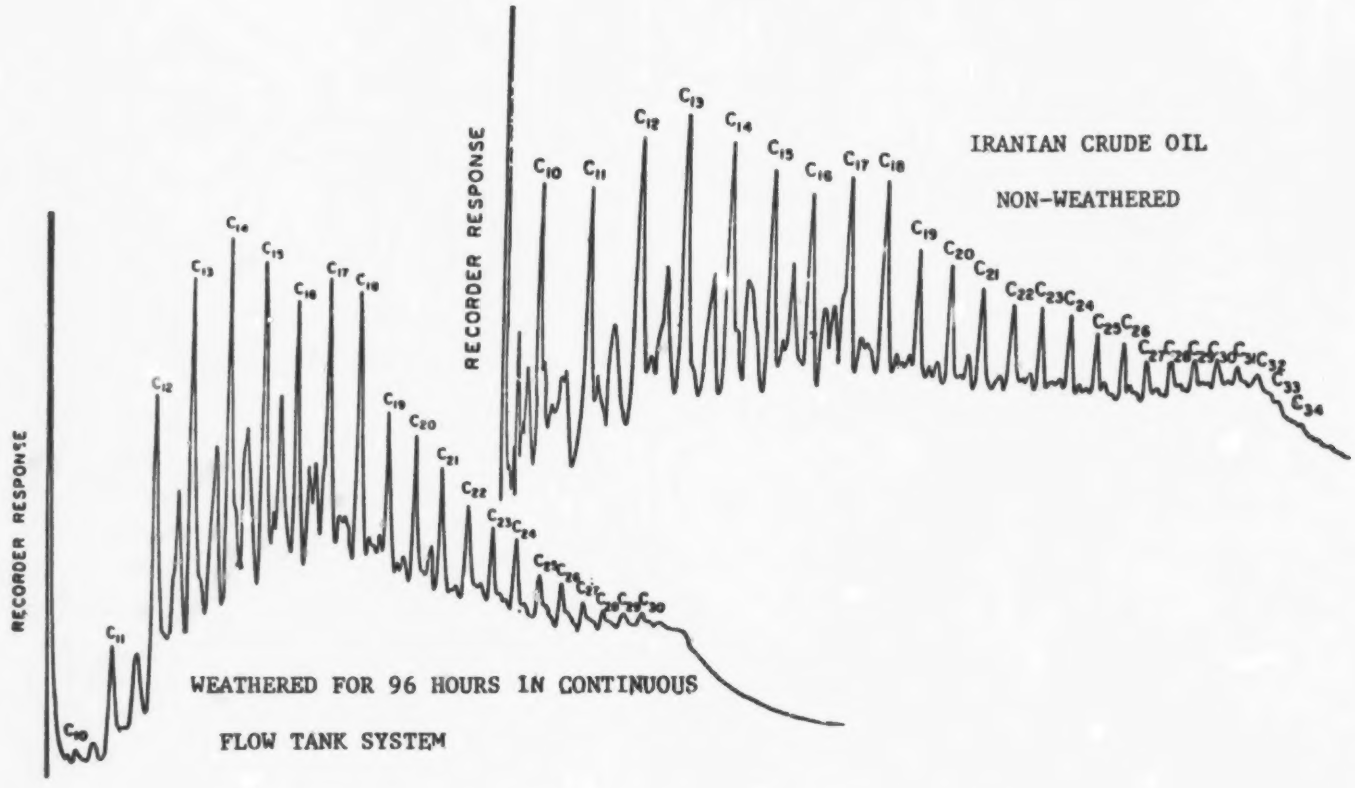

Fig. 5. Chromatograms of Iranian crude oil before and after weathering for 96 hours in continuous flow tank system.

BIOACCUMULATION

Colonies of the octocoral, Heteroxenia fuscescens, exposed to 10 ml/l crude oil for 96 hours showed marked uptake in the C_{11}-C_{15} range and also in the region above C_{23} (Figure 6). Extreme care was taken that colonies were not in actual contact with oil film. Colonies subjected to only 3 ml/l oil for 96 hours showed little uptake in the C_{11}-C_{15} range, but the hydrocarbon distribution above C_{23} remained in close agreement with that of the higher application level. It is possible that the reduced C_{11}-C_{15} fraction in tissues may be due to evaporative loss of those fractions prior to incorporation.

The amount of oil taken up by the most contaminated corals, i.e. those exposed to 10 ml/l oil for 96 hr was less than 1% of the total hydrocarbon content of control corals. We conclude that corals, although comparatively resistant to crude oil under simulated natural conditions, are capable of incorporating oil from the surrounding medium into its tissues. Bioaccumulation and long-term retention of fuel oil components by shellfish is documented by Blumer (1). However, the metabolic implications of Blumer's observations and our own findings are imperfectly understood and appear to merit further investigation.

RECOMMENDATIONS

On the basis of studies summarized herein as well as available personnel and equipment, we suggest a broadening of the oil research program at the Elat Marine Biological Laboratory during the coming year in the areas of bioassay, chemical residues, behavior, and field monitoring studies. Specifically, we recommend the following:

Bioassay

Laboratory testing of chemical oil dispersants and other oil counteractants, both alone and in combination with target oils at realistic application levels, should continue using representative species of Red Sea biota. Bioassays are to be conducted under conditions of continous flow in containers of sufficient height (2.0 m is suggested) to account for possible depth protective effects. Test species would include various life history stages of hermatypic and non-hermatypic corals, perciform teleosts, bivalve molluscs, decapod crustaceans, echinoid echinoderms, and conceivably

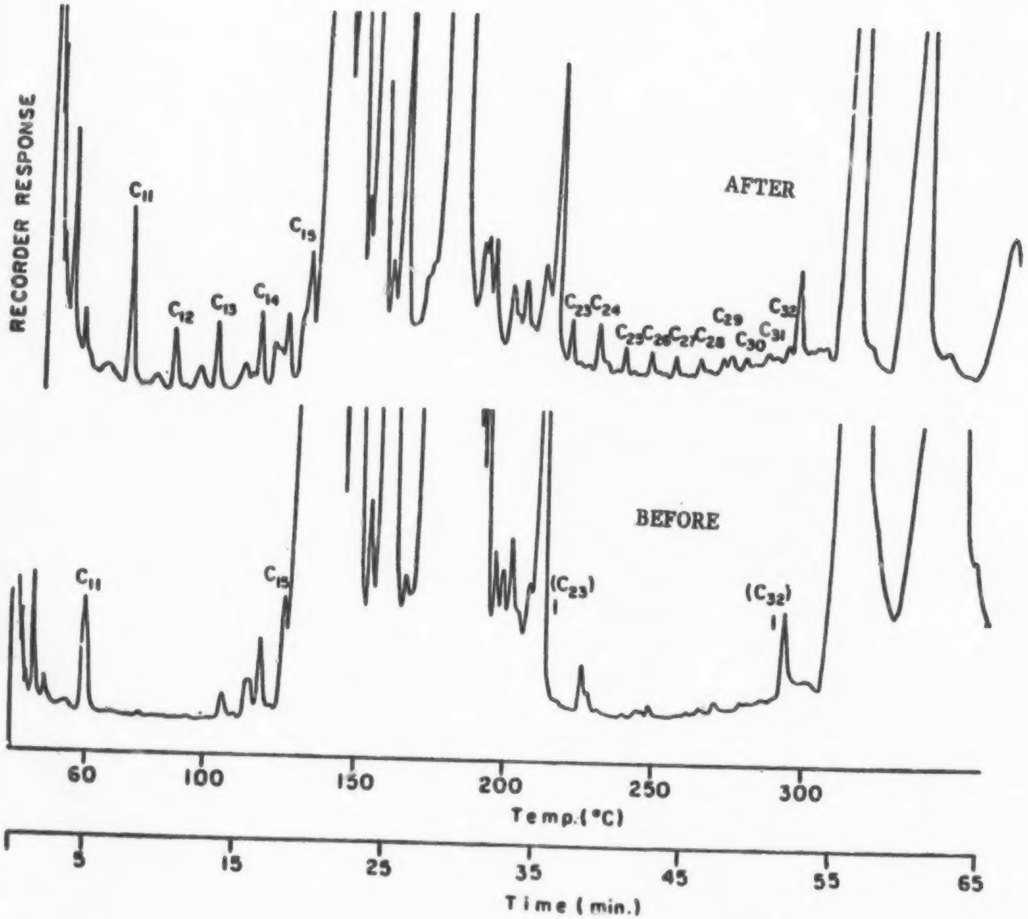

Fig. 6. Gas chromotograms of Heteroxenia fuscescens before and after exposure to 10 ml/1 Iranian crude oil for 96 hours.

other groups. Availability of toxicants in the media to assay organisms should be ascertained using appropriate chemical procedures. Research thrust in this problem area would be on long-term biocidal properties of test compounds, as modified by physiochemical factors and dosing rates.

Chemical Residues

Rate and extent of bioaccumulation of crude oils, oil counteractants, and mixtures should be determined under simulated field conditions. This would include data on accumulation from the medium and through marine food chains using gas chromatography and other techniques. Research emphasis should be on hydrocarbon residues from flesh of commercially important species, especially teleosts, but also crustaceans and bivalve molluscs.

Behavior Studies

Impact of sublethal concentrations of crude oils and oil-counteractant mixtures to biota, with special reference to inshore fauna, should be evaluated during exposure and afterwards. We suggest that priority be given to type studies of attractance-repellence, stamina and predator avoidance, feeding behavior, long-term effects on fecundity, and disease resistance.

Field Studies

We recommend that a live well monitoring capability be established near oil-unloading terminals, and other potential point sources. At this time adult lobsters appear promising as suitable and sensitive indicators. Also, dispersant compounds proven under laboratory conditions to be low in toxicity, comparatively degradable, and efficient oil counteractants should be promptly field tested on typical coral reef ecosystems utilizing suitable containment materials. For all studies, we recommend a chemical residue analytical capability.

ACKNOWLEDGEMENTS

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ADSORPTION OF ORTHOPHOSPHATE ON BOROSILICATE AND "CITRATE OF MAGNESIA BOTTLES" POLYETHYLENE AND POLYVINYL SURFACES IN A DISTILLED WATER AND SEAWATER MATRIX

D. F. Krawczyk and M. W. Allen *

The chemical analysis of nutrients in marine and estuarine waters has been going on since before the turn of the century. A glance at the oceans where upwelling liberates an abundance of nutrients will provide the realization that the oceans are an important source of food supply. The task of analyzing marine and estuarine waters accurately and precisely is the responsibility of the oceanographic chemist who has historically accomplished his analytical work on site. Those tests that could be conducted on shipboard were performed immediately. Thus, an oceanographic vessel would carry burets, pipettes, and meters that the chemist needed to perform analyses. If for some reason an analysis could not be performed immediately, then the techniques of preservation were freezing and cold storage until analysis could be completed.

Today, the oceanographic vessel can be equipped with probes and automated analytical equipment allowing precise analysis to be accomplished on site (1,2). However, insight to some of the problems that may be encountered on oceanographic vessels can be gleaned from a passage from Scientific American's special "Oceans" issue.

> Being an oceanographer is not quite the same as being a professional sailor. Oceanographers have the best of two worlds--both the sea and the land. Yet many of them, like many sailors, find it extraordinarily satisfying to be far from the nearest coast on one of the small, oily and uncomfortable ships of their trade, even in the midst of a vicious storm, let alone on one of those wonderful days in the Tropics when the sea and air are smiling and calm.
>
> I think the chief reason is that on shipboard both the past and future disappear. Little can be done to remedy the mistakes of yesterday; no planning for tomorrow can reckon with the unpredictability of ships and the sea. To live in the present is the essence of being a seaman.

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> The work of an oceanographer, however, is inextricably related to time. To understand the present ocean he must reconstruct the present ocean, he must reconstruct his history, and to test and use his understanding he needs to be able to predict--both what he will find by new observations and future events in the sea. (3).

The relevance of the above comments to the present study lies mainly in the framework and magnitude of "time" it reveals for the meaningful interpretation of marine samples. A mechanical breakdown of equipment aboard ship would require back-up systems for storage of samples until analysis was possible. In facing the problem of storage consideration must be given to the potential problem of adsorption, especially in the case of orthophosphate. Heron (4), Hassenteufeul (5), and Ryden (6) have all reported an adsorption of phosphate on glass, polyethylene, polypropylene, and polycarbonate. The problem presented a variety of situations which were attributed to adsorption of phosphate onto surfaces. The problem of adsorption, it should be noted, will affect the accuracy of the analysis but not necessarily the precision.

EXPERIMENTAL

This study concerns itself with the measurement of orthophosphate in distilled water and seawater after contact with borosilicate glass, "citrate of magnesia bottle", thin wall polyethylene Cubitainer®, commercial grade wall polyethylene and polyvinyl chloride bottles as sample holding containers.

The newly purchased, first-time borosilicate glass and soda lime glass (citrate) bottles, were washed in phosphate-free detergent, rinsed with deionized water, rinsed with double distilled water and dried. The unused polyethylene containers and the PVC bottles were used without any pretreatment. Five replicates of sample were analyzed. A second set of five replicates received 40 mg/l of mercuric chloride. To a third set of five replicates orthophosphate was added. The addition was made with a microliter pipette from a concentrated orthophosphate solution. A fourth set of five replicates received an addition of mercuric chloride to provide a 40 mg/l concentration and an incremental addition of orthophosphate again spiked via microliter pipette. An analysis of waters used is presented in Table 1.

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TABLE 1. Characteristics of Water

Parameter	Concentration	Distilled Water	Seawater
pH	Units	6.8	7.6
Conductivity	mhos*	0.6	33.3*
Total Inorganic Carbon	mg/l	<1	26
Total Organic Carbon	mg/l	< .3	1.2
Kjeldahl Nitrogen	mg/l	< .01	.2
Nitrate Nitrogen	μg/l	<1	200
Nitrite Nitrogen	μg/l	<1	2
Total Phosphate Phosphorus	μg/l	<1	35
Orthophosphate Phosphorus	μg/l	<1	35
Total Chromium	μg/l	<1	<5
Total Cobalt	μg/l	<1	< 20
Total Copper	μg/l	1	15
Total Iron	μg/l	20	380
Total Lead	μg/l	<5	<25
Total Manganese	μg/l	<1	46
Total Zinc	μg/l	1	7

*Salinity in parts per thousand

The rationale in using off-the-shelf bottles was an attempt to model a shipboard situation where a mechanical breakdown would force the use of alternative systems. An assumption is made that time would not permit a thorough preparation for use of sample bottles.

The waters were analyzed for orthophosphate phosphorus using the automated phosphomolybdate procedure. Details of the analysis are available (7,8,9). A salt correction was made where analysis of marine waters was conducted (10). A single analysis was performed on each bottle. The data for orthophosphate analysis of distilled water and seawater is presented in Tables 2-8.

The results presented in Table 2 on borosilicate glass when compared to data of Table 1 indicate that phosphate or some constituent providing a positive phosphate level is being leached from glassware very quickly. Some indication of release of phosphate was also evidenced in the "Citrate of Magnesia Bottle" but the data do not appear as convincing as in the case of the borosilicate glass. The examination of these data after the seventh day required analysis in triplicate on a selected number of bottles on the eighth day. The results of the triplicate analysis are presented in Table 4. These data indicate that there were some problems in the data reported on the sixth day. Thus, the analytical quality control (AQC) aspects of presented data would allow for deletions of the data from the sixth day. It also lends credence to the assumption that the numbers obtained from the borosilicate glass portions of the study are meaningful.

The consistency of the data in Table 5, with the exception of the data portion after six days which was rejected based on AQC, provides still more evidence that the values in Table 2 have some meaning. A second group of borosilicate bottles was washed with phosphate-free detergent, rinsed, dried, rerinsed with double distilled water and dried. The data for this second set are presented in Table 6. Triplicate analyses on a selected number of bottles were conducted after the second day. The purpose of the analyses after 2 days is to provide validity for the use of a single analysis per bottle concept. The second rinsing with distilled water seemed to have provided some improvement in leaching of some component providing a positive orthophosphate level. A hydrochloric acid wash followed by a phosphate-free detergent wash followed by a distilled water rinse followed by drying provided orthophosphate analyses that were similar to blanks and were less than 1μg/l.

Two types of polyethylene bottles were used in this study. A bottle identified as a polyethylene Cubitainer® of one liter size (produced from hot melted polyethylene

TABLE 2. Borosilicate Glass - 500 ml size
Distilled Water Matrix

Bottle Number	Treatment	Zero time µg/l Orthophosphate Phosphorus	After 2 days Contact µg/l Orthophosphate Phosphorus	After 6 days Contact µg/l Orthophosphate Phosphorus	After 7 days Contact µg/l Orthophosphate Phosphorus
1	None	1	3	5	4
2	None	1	4	2	3
3	None	8	14	1	17
4	None	3	2	8	9
5	None	3	1	4	3
6	$HgCl_2$ *Added	2	2	21	3
7	$HgCl_2$ *Added	12	2	12	2
8	$HgCl_2$ *Added	3	3	4	3
9	$HgCl_2$ *Added	15	4	5	4
10	$HgCl_2$ *Added	8	10	8	3
11	Spike **	17	9	4	4
12	Spike **	12	8	6	14
13	Spike **	6	7	9	9
14	Spike **	10	6	10	9
15	Spike **	9	9	9	10
16	Spike + $HgCl_2$ Added	7	13	9	10
17	Spike + $HgCl_2$ Added	13	8	8	11
18	Spike + $HgCl_2$ Added	11	9	9	8
19	Spike + $HgCl_2$ Added	9	8	8	9
20	Spike + $HgCl_2$ Added	14	6	14	15

*$HgCl_2$ = 40 mg/l in sample
**Spike = 4 µg/l P

TABLE 3. "Citrate of Magnesia Bottle"

Distilled Water Matrix

Bottle Number	Treatment	Zero time µg/l Orthophosphate Phosphorus	After 2 days Contact µg/l Orthophosphate Phosphorus	After 6 days Contact µg/l Orthophosphate Phosphorus	After 7 days Contact µg/l Orthophosphate Phosphorus
21	None	3	1	14	12
22	None	6	1	9	2
23	None	3	1	10	2
24	None	2	1	1	1
25	None	3	1	1	1
26	$HgCl_2$ Added*	1	1	1	1
27	$HgCl_2$ Added*	0	1	1	2
28	$HgCl_2$ Added*	0	1	1	2
29	$HgCl_2$ Added*	0	1	1	2
30	$HgCl_2$ Added*	2	1	1	2
31	Spike Added**	-	7	1	2
32	Spike Added**	-	7	1	6
33	Spike Added**	-	7	1	15
34	Spike Added**	-	6	5	11
35	Spike Added**	-	14	6	9
36	Spike + $HgCl_2$ Added	-	5	5	7
37	Spike + $HgCl_2$ Added	-	6	5	8
38	Spike + $HgCl_2$ Added	-	6	12	8
39	Spike + $HgCl_2$ Added	-	6	6	8
40	Spike + $HgCl_2$ Added	-	6	6	7

*$HgCl_2$ = 40 mg/l in sample
**Spike = 4 µg/l

TABLE 4. Triplicate Analysis on Selected Bottles
After the Eighth Day of Contact in a
Distilled Water Matrix

Bottle Type	Bottle Number	Treatment	Seventh Day (a)	Eighth Day Replicate Analysis µg/l Orthophosphate First	Second	Third
Borosilicate Glass	3	None	17	15	15	14
Borosilicate Glass	6	$HgCl_2$	3	1	1	2
Borosilicate Glass	11	Spike*	4	10	10	11
Borosilicate Glass	16	Spike*+ $HgCl_2$	10	8	8	9
**CMB Glass	21	None	12	1	1	2
**CMB Glass	28	$HgCl_2$	2	1	1	1
**CMB Glass	31	Spike*	2	5	5	4
**CMB Glass	38	Spike*+ $HgCl_2$	8	4	4	5
Polyethylene	Cubitainer^R	None	2	1	1	1
Polyethylene	Cubitainer^R	$HgCl_2$	1	1	1	1
Polyethylene	Cubitainer^R	Spike*	4	7	5	5
Polyethylene	Cubitainer^R	Spike*+ $HgCl_2$	7	7	7	7
Polyethylene	Molded Bottle	None	1	1	1	1
Polyethylene	Molded Bottle	$HgCl_2$	1	1	1	1
Polyethylene	Molded Bottle	Spike*	6	5	5	4
Polyethylene	Molded Bottle	Spike*+ $HgCl_2$	7	6	5	4
PVC	Molded Bottle	None	2	1	1	1
PVC	Molded Bottle	$HgCl_2$	1	1	1	1
PVC	Molded Bottle	Spike*	5	4	3	2
PVC	Molded Bottle	Spike*+ $HgCl_2$	5	5	3	2

*Spike level = added 4 µg/l of orthophosphate
** CMB = "Citrate of Magnesia Bottle"
Cubitainer^R Registered trademark of Hedwin Corp.
(a) Data from seventh day is for comparative purposes

TABLE 5. Plastic Bottles

Distilled Water Matrix

Bottle Type	Treatment	Zero Time Mean of Five Replicates µg/l Orthophosphate Phosphorus	After 2 Days Mean of Five Replicates µg/l Orthophosphate Phosphorus	After 6 days Mean of Five Replicates µg/l Orthophosphate Phosphorus	After 7 Days Mean of Five Replicates µg/l Orthophosphate Phosphorus
Polyethylene*	None	<1	2	<1	1
Polyethylene*	$HgCl_2$	<1	1	6	1
Polyethylene*	Spike**	6	5	6	7
Polyethylene	Spike**+ $HgCl_2$	5	7	6	7
Polyethylene (a)	None	<1	<1	<1	1
Polyethylene (a)	$HgCl_2$	1	<1	3	2
Polyethylene (a)	Spike **	3	6	3	6
Polyethylene (a)	Spike**+ $HgCl_2$	3	6	3	7
PVC (b)	None	<1	<1	<1	2
PVC (b)	$HgCl_2$	<1	1	3	1
PVC (b)	Spike**	3	5	4	5
PVC (b)	Spike**+ $HgCl_2$	2	5	5	7

*Polyethylene one liter Cubitainer^{R} - registered trademark Hedwin Corp.
**Spike Level = added 4 µg/l of orthophosphate
(a) Polyethylene molded bottle
(b) Polyvinylchloride molded bottle.

TABLE 6. Borosilicate Glass Special Rinse Distilled Water Matrix

Bottle Number	Treatment	Zero Time µg/l Orthophosphate Phosphorus	After 1 Day µg/l Orthophosphate Phosphorus	After 2 Days µg/l Orthophosphate Phosphorus	After 2 Days µg/l Orthophosphate Phosphorus	After 2 Days µg/l Orthophosphate Phosphorus
101	None	2	4	2	1	1
102	None	1	3			
103	None	9	3			
104	None	2	3			
105	None	1	7			
106	$HgCl_2$	4	5	2	2	1
107	$HgCl_2$	4	4			
108	$HgCl_2$	2	4			
109	$HgCl_2$	6	5			
110	$HgCl_2$	6	4			
111	Spike**	5	7			
112	Spike*	8	8	4	3	2
113	Spike*	7	5			
114	Spike*	6	5			
115	Spike*	6	5			
116	$HgCl_2$ + Spike*	4	-			
117	$HgCl_2$ + Spike*	7	9			
118	$HgCl_2$ + Spike*	7	5	5	5	3
119	$HgCl_2$ + Spike*	1	8			
120	$HgCl_2$ + Spike*	1	7			

*Spike level = added 4 µg/l of orthophosphate.

sheets with fused seams) was one of the polyethylene bottles used. A molded polyethylene bottle of 250 ml volume was the second polyethylene bottle used. A clear polyvinyl chloride widemouthed, molded bottle of one liter size was the sample container noted as polyvinyl chloride bottle.

The data in Tables 4 and 5 indicate little change in orthophosphate in a distilled water matrix during the seven-day period.

The next experiment dealt with potential losses of orthophosphate as a function of time in a seawater matrix. The seawater used in this study was taken from a well-aged composite held in a tank filled to 20 percent from a January, 1973 collection and 80 percent from an April, 1973 collection at Depoe Bay, Oregon. As in the first study, five replicates in four groups (natural water, water + $HgCl_2$, water + spike, water + spike + $HgCl_2$) were prepared with the single difference being the concentration of the orthophosphate after 2 days and after 16 days. The results of the analyses are present in Table 7. The observed differences between the data from the second to sixteenth day are presented in Table 8. It should be pointed out in Table 7 that the spike level was added to a distilled water matrix and used as a control. The borosilicate glass bottles used for control were previously acid washed, rinsed with distilled water and dried. A second set of acid washed borosilicate glass bottles was used for the seawater matrix experiment. The data for the second set is also shown in Table 7 with analysis being conducted only on the sixteenth day.

DISCUSSION

The sample bottles were kept at laboratory room temperature in laboratory light and handled in the same manner as any sample collected for analysis. No special shaking was provided for the bottles.

The data in Tables 2, 4, and 6 indicate the borosilicate bottles must be cleaned with hydrochloric acid and rinsed well with distilled water before use as collection bottles. When the quality of water sample approaches that of distilled water, a significant error in release of materials from borosilicate bottles may occur. The interesting point in Table 5 is the use of off-the-shelf (unused) polyethylene and polyvinyl chloride bottles and negligible losses observed for orthophosphate phosphorus in distilled water.

With a seawater matrix, little change was observed in borosilicate glass bottles, citrate of magnesia glass bottles, and polyvinyl chloride bottles. Significant changes were observed in polyethylene bottles after sixteen

TABLE 7. Seawater Matrix

Bottle Type	Treatment	After 2 Days Contact Mean of Five Replicates μg/l Orthophosphate Phosphorus	Standard Deviation	After 16 Days Contact Mean of Five Replicates μg/l Orthophosphate Phosphorus	Standard Deviation
Borosilicate Glass	None	33.6	4.6	44.0	4.0
	$HgCl_2$	39.4	4.8	50.4	6.8
	Spike (a)	59.6	3.6	63.2	1.1
	$HgCl_2$ + Spike(a)	66.2	3.5	70.4	2.9
"CMB"	None	34.4	.5	36.0	.71
	$HgCl_2$	34.2	.8	37.8	.1
	Spike (a)	56.4	1.4	57.3	.6
	$HgCl_2$ + Spike (a)	58.0	1.4	59.4	.4
Polyethylene Cubitainer^R	None	34.1	.2	3.4	2.3
	$HgCl_2$	34.9	.3	30.7	4.3
	Spike (a)	59.1	.6	19.6	4.3
	$HgCl_2$ + Spike (a)	59.2*	1.0*	59.6	2.1
Polyethylene Molded Bottles	None	32.6	.9	1.4*	.6*
	$HgCl_2$	34.5	.5	38.5	.4
	Spike (a)	61.6	1.2	29.8	1.7
	$HgCl_2$ + Spike(a)	62.6	1.1	64.8	1.1
PVC Molded Bottles	None	32.8	.4	35.4	.9
	$HgCl_2$	33.7	.4	38.0*	.1*
	Spike (a)	61.3	.5	61.4	.7
	$HgCl_2$ + Spike (a)	58.9	3.9	61.3	3.3
Dist. H_2O in Borosilicate	None	1.0		1.0	-
	Spike (a)	20.4	2.8	20.8	1.8
Borosilicate Glass Acid Washed	None			38.2	2.4
	$HgCl_2$			35.4	.5
	Spike (a)			68.2	3.0
	$HgCl_2$ + Spike (a)			60.4	.9

"CMB" Citrate of Magnesia Bottle
(a) Spike level 25.1 μg/l ± 3.1 μg/l (n=20)
* N=4
Cubitainer^R registered trademark of Hedwin Corp.

TABLE 8. Magnitude of Change Noted Between 2nd & 16th Days
Seawater Matrix

Container	Treatment	Change From Day 2 - Day 16 μg/l	Pooled Standard Deviation μg/l
Borosilicate Glass	None	+10.4	4.3
	$HgCl_2$	+11.0	5.9
	Spike (a)	+ 3.6	2.7
	$HgCl_2$ + Spike (a)	+ 4.2	3.2
"CMB" Glass	None	+ 1.6	.6
	$HgCl_2$	+ 3.6	.6
	Spike (a)	+ .9	1.1
	$HgCl_2$ + Spike (a)	+ 1.4	1.0
Polyethylene Cubitainer^R	None	-30.7	*
	$HgCl_2$	- 4.2	3.0
	Spike (a)	-39.5	*
	$HgCl_2$ + Spike (a)	.4	1.7
Polyethylene Molded Bottle	None	-31.2	*
	$HgCl_2$	+ 4.0	.5
	Spike (a)	-31.8	*
	$HgCl_2$ + Spike (a)	+ 2.4	1.1
Polyvinyl Chloride Molded Bottle	None	+ 2.6	.7
	$HgCl_2$	+ 4.3	.2
	Spike (a)	+ .1	.6
	$HgCl_2$ + Spike (a)	+ 2.4	3.6

"CMB" Citrate of Magnesia Bottle
(a) Spike level 25.1 μg/l ± 3.1 μg/l (n=20)
* Pooled standard deviation not computed because of substantial change in data from second to sixteenth day.
Cubitainer^R Registered trandmark of Hedwin Corp.

days both in untreated seawater and seawater receiving incremental additions of orthophosphate phosphorus. No significant changes were observed in polyethylene bottles that contained 40 mg/l of mercuric chloride either in the seawater or orthophosphate-spiked seawater. No attempt was made to identify the cause for loss. The data in Table 8 point out an orthophosphate phosphorus loss level of 30.7 to 39.5 ug/l. Although the surface area in a Cubitainer® was four times the surface area in a molded polyethylene bottle, these differences appear to be a constant, or at least within experimental error.

Two theories can be advanced: (a) biological uptake with inhibition in glass or polyvinyl chloride bottles, or (b) preferential adsorption of mercuric chloride upon adsorbing sites available on the polyethylene surface which would inhibit adsorption of orthophosphate ions. Of the two theories the biological uptake theory appears less likely because of the loss of such a significant level of orthophosphate.

It is interesting to note that little change was observed in the polyvinyl chloride bottles in orthophosphate concentrations both in unpreserved and preserved samples. An effective AQC program permitted the rejection of questionable data.

SUMMARY

Adsorption or loss of orthophosphate in glass bottles does not seem to be a problem at low orthophosphate concentration in a distilled water matrix up through seven days. However, glass bottles must have proper acid treatment to be satisfactory containers for low phosphate water samples because of leaching of a constituent that provides a positive phosphate reaction.

In seawater, losses of orthophosphate phosphorus were noted after a sixteen day period in polyethylene bottles. If mercuric chloride is added to seawater, polyethylene bottles can be used for holding samples for orthophosphate analysis without any pretreatment (e.g. acid washing, washing and rinsing). Polyvinyl chloride bottles can be used as seawater sample storage containers as received from the supplier without any pretreatment.

Borosilicate glass bottles must be acid washed and thoroughly rinsed before holding samples for orthophosphate analysis.

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DEVELOPMENT OF A STANDARD MARINE ALGAL ASSAY PROCEDURE FOR NUTRIENT ASSESSMENT

D. T. Specht and W. E. Miller*

INTRODUCTION

The need for a rapid, accurate means to assess water quality in marine and estuarine situations is no less urgent than for freshwater systems. Estuaries are particularly valuable and sensitive resources (1,2,), for which no standardized primary producer bioassay for water quality exists. Bioassays have been developed for very specific purposes, but most are narrow in design and require highly specialized personnel to operate and evaluate (3, 4, 5, 6, 7, 8, 10, 11, 12, 14).

Inasmuch as estuaries are the "nurseries" of virtually all commercially important shellfish, fish and their food organisms, it becomes vitally important to be able to accurately assess their capabilities for primary productivity and to determine and predict the impact of the addition of various nutrients and wastewaters upon that productivity (15, 5, 16, 17, 11, 13, 18, 23, 19, 20, 21, 22, 42).

For reasons of simplicity and consistency, the protocol for the marine algal assay follows closely that of the freshwater algal assay -- "Algal Assay Procedure: Bottle Test" (39) published in August 1971. This is particularly true of the physical facilities required, incubation conditions and methodology.

ESTABLISHMENT OF CRITERIA FOR BIOASSAY ORGANISM

Unlike freshwater systems, estuaries in particular pose the problem of interpretation of varying salinities on the effect of nutrient concentration biomass production. While no algal species was thought to be unaffected by varying salinity, the most ideal candidate would be one that responded in a linear and predictable manner to such changes. Accordingly, this criterion was judged to be of overriding importance. A species was desired that would also be genetically stable, reproduce asexually, and would not adhere to the walls of the flasks or tend to clump.

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Other major problem areas to be considered were the array of factors that govern the consistency of growth response such as light intensity, temperature, influence of pH, relative size of inoculum as well as selection and modification of a standard stock medium in which the intracellular nutrient carryover in the inoculum would be minimal.

A significant consideration is the ease of evaluation or measurement of the algal biomass produced. For purposes of consistency, all results would be converted to equivalent dry weight, as compared with standard gravimetric methods. (39).

SELECTION OF CANDIDATE TEST SPECIES

Considering the established criteria, candidate algal species were selected on several bases looking for three distinct classes of algae. For biostimulation, one should consider pollution tolerant algae, such as the green algae Dunaliella tertiolecta, and Nannochloris atomus. For toxicity or inhibition considerations, one should look toward typical crustacean or bivalve food organisms such as Isochrysis lutheri, Cyclotella menenghiana, and Thalassiosira pseudonana. For situations in which nitrogen is limiting, a nitrogen fixing blue-green alga should be considered. Some species to be examined are Coccochloris elabens and Trichodesmium sp. (24).

These algae represent three taxonomic classes: the green algae, blue-green algae, and diatoms. Thus far, most of the work has centered around the green flagellate, Dunaliella tertiolecta Butcher (DUN clone), kindly provided by Dr. John Ryther, Woods Hole Oceanographic Institution. This organism has shown the most linear response for every parameter examined (25). An additional advantage of Dunaliella is that it requires no outside source of vitamins (26). Comparisons of field samples have been conducted with Thalassiosira pseudonana Hasle and Heimdal, but more work is needed on salinity - nutrient interactions. After the needed information on the diatom is assembled, more intensive efforts will be made in search of a suitable blue-green alga.

DEVELOPMENT OF STANDARD MEDIA RESPONSE CURVES

The initial work with Dunaliella was performed using a modified Burkholder's Artificial Sea Water (ASW) (27). This is one of several potentially amenable media which will support algal growth at relatively low nutrient levels (28, 29), Table 1.

A. Salinity Tolerance and Salt Interaction

Although it was assumed that all algae would be affected to some degree by the salinity of the media. it was desirable to find the alga with the most linear response and tolerance to manipulation of salinity, primarily between 5‰ and 35‰. In order to establish linearity of response, levels of salinity chosen were 5, 8, 12, 16, 20, 24, 30 and 35‰, Figure 1. The initial tests were made with rather high levels of nutrients; but later, nutrient response trials showed essentially the same linearity at much lower levels. The data thus produced provided the basis for a three-dimensional plot, such as Figure 2, which portrays response "surfaces" according to salinity-nutrient interactions. These responses appear to be predictable, within limits, Figure 2.

TABLE 1

MARINE ALGAL ASSAY PROCEDURE SUMMARY: Eutrophication and Lake Restoration Branch, PNERL, NERCC, EPA

I. TEST ORGANISM: *Dunaliella tertiolecta* Butcher (DUN clone) available from the Eutrophication and Lake Restoration Branch, PNERL, EPA. 200 SW 35th St., Corvallis, OR 97330; or WHOI.

II. ENVIRONMENTAL CONDITIONS: In general, follow exactly the ALGAL ASSAY PROCEDURE BOTTLE TEST (NERP, EPA, August, 1971) with the following exceptions:

TEMPERATURE:	18 - 20°C
SAMPLE SIZE:	100 ml in a 500 ml Erlenmyer flask
INOCULUM:	Inoculate with 1 ml washed (w/ 20 ‰ ASW less N & P, by serial centrifugation) 5-8 day old culture at a concentration of 10,000 cells/ml (to give a final concentration in the flask of 100 cells/ml, equivalent to approximately 0.03 mg dry wt/l).

III. BASAL MEDIUM: Modified Burkholder's Artificial Seawater (ASW) with NAAM levels of the following nutrients; N, P, Fe, and NaEDTA. Use Analytical Reagent or Reagent Grade chemicals, and double glass distilled water.

Compound		grams/l	grams/4l
NaCl		23.48	93.92
Na_2SO_4		3.92	15.68
$NaHCO_3$		0.19	0.76
KCl		0.66	2.64
KBr		0.096	0.384
H_3BO_3		0.026	0.104
$MgCl_2 \cdot 6H_2O$		10.61	42.44
$SrCl_2 \cdot 6H_2O$		0.04	0.16
$CaCl_2 \cdot 2H_2O$		1.469	5.876
H_2O	to	1,000 ml	4,000 ml

filter through prewashed 0.45 µm membrane filter.

FOR DILUTION TO VARIOUS SALINITIES: (4 liter batches)

Salinity, ‰	ASW stock, l	H_2O, l (glass distilled)
35	4.0	0.0
30	3.428	0.571
24	2.742	1.257
20	2.285	1.714
16	1.828	2.171
12	1.371	2.628
8	0.914	3.085
5	0.571	3.428

For any given final salinity, mix well, adding the following NAAM levels of nutrients:

$NaNO_3$	102 mg/4l batch (4.2 mg N/l)
K_2HPO_4	4.176 mg/4l batch (0.186 mg P/l)
NaEDTA	1200 μg/4l batch (300 μg/l)

*NAAM trace metal mix (minus $FeCl_3$)

filter through .45 μm membrane filter,

add AFTER filtration, sterilized $FeCl_3$, 384 μg/4l batch (33.05 μg Fe/l).

* Add the following: 0.0928 g H_3BO_3; 0.208 g $MnCl_2 \cdot 4H_2O$; 0.016 g $ZnCl_2$; 0.714 mg $CoCl_2 \cdot 6H_2O$; 0.0107 mg $CuCl_2 \cdot 2H_2O$; 3.63 mg Na_2MoO_4 $2H_2O$; make up to 500 ml, adding 1 ml of this concentrate to each liter of media.

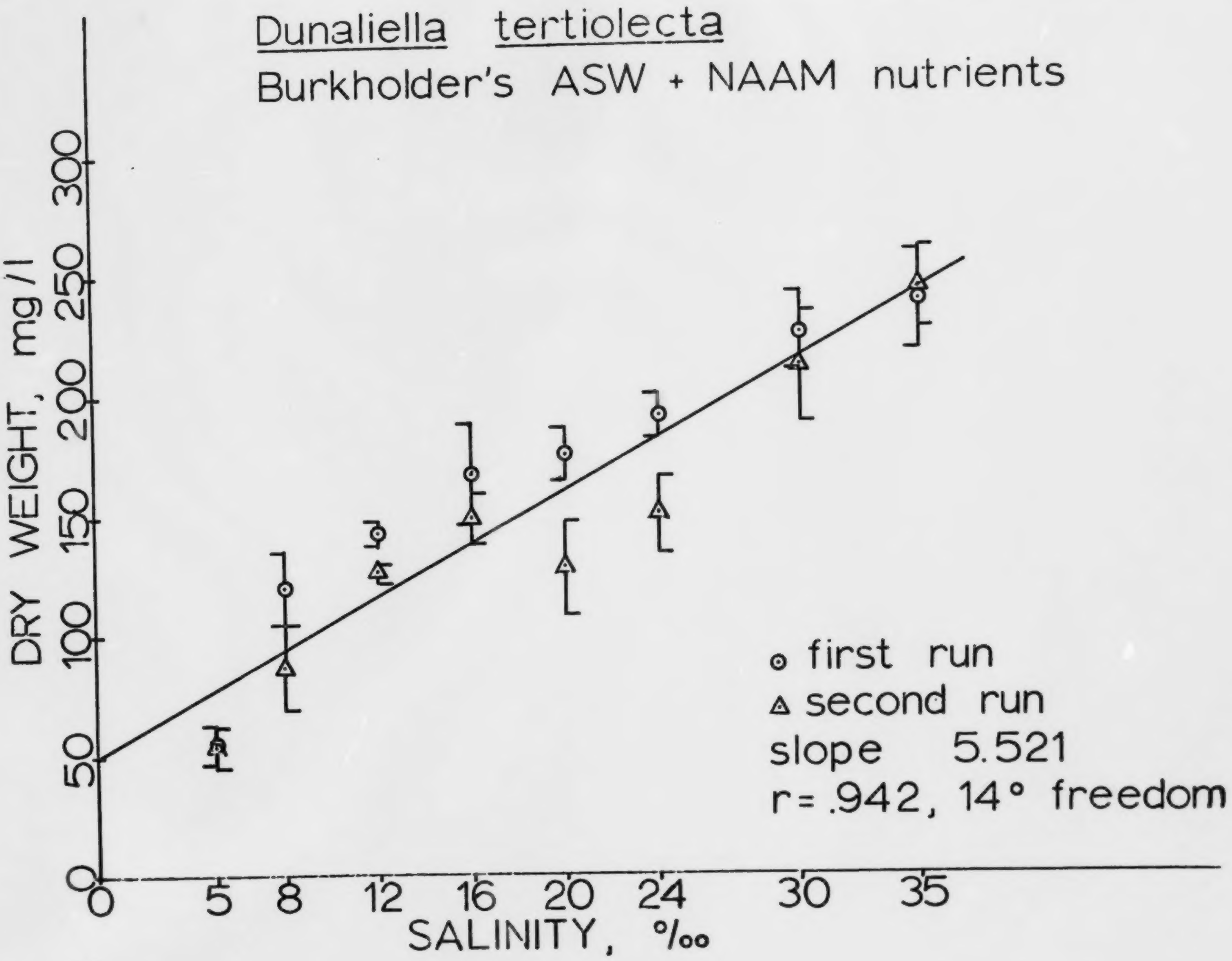

Dunaliella tertiolecta
Burkholder's ASW + NAAM nutrients
DRY WEIGHT, mg/l
0
50
100
150
200
250
300
SALINITY, ‰
0
5
8
12
16
20
24
30
35
○ first run
△ second run
slope 5.521
r = .942, 14° freedom

Fig. 1

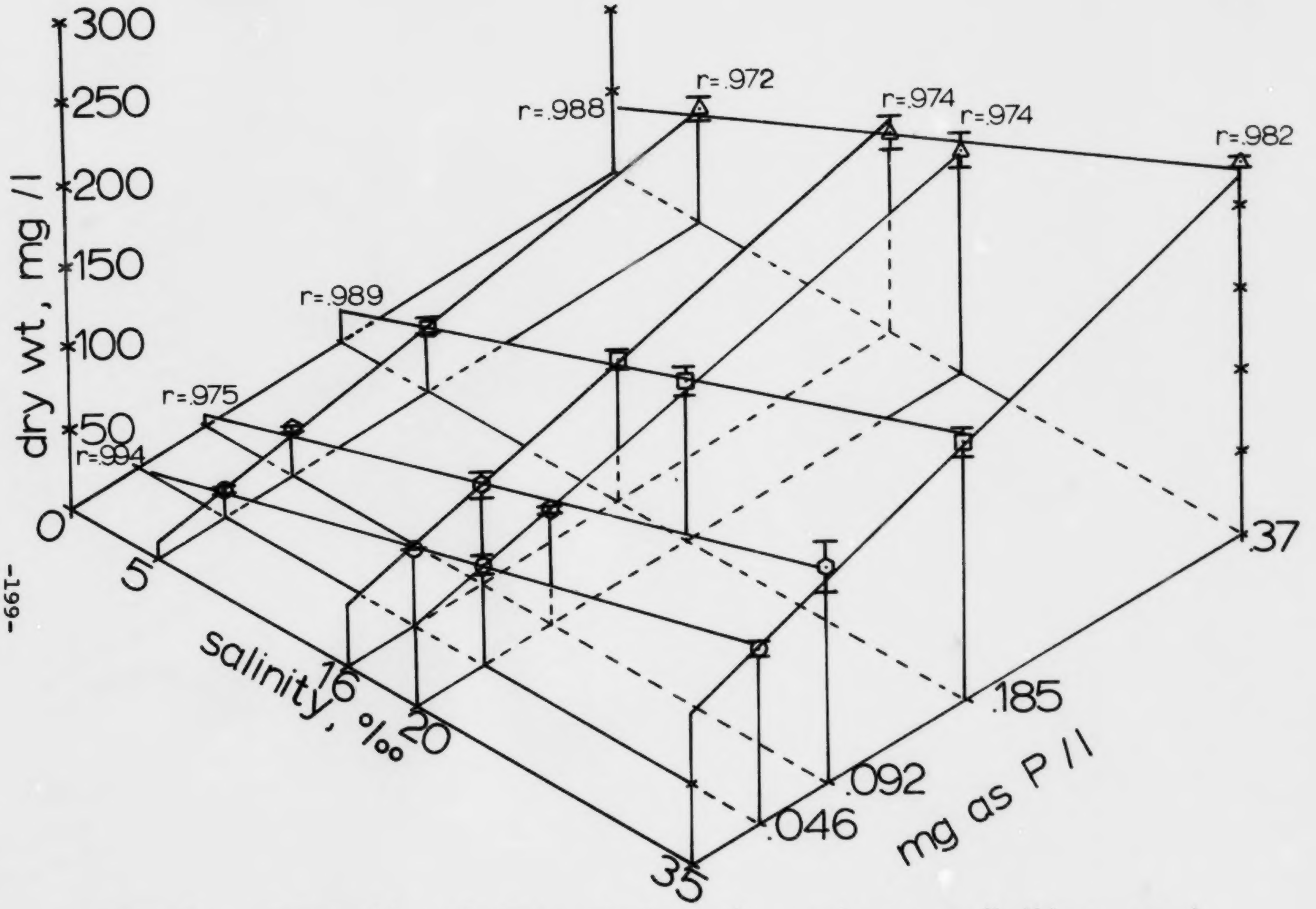


Fig. 2 Growth of Dunaliella at various salinities and phosphorus concentrations.

B. Temperature and Light

Several trials were made at 24± 2°C, the standard temperature used in the freshwater Algal Assay Procedure. However, responses were both irregular and too rapid for accurate assessment; and replication was particularly poor. A temperature of 18°C was found to yield satisfactory results in these same terms (25).

Light levels of 400 ft-c ± 10 percent (1300 $\mu w/cm^2$; 4300 lux) proved satisfactory for Dunaliella, and no reason was seen to change this from the freshwater Algal Assay Procedure.**

The work so far with Thalassiosira has been done at 20±2°C, with no shaking, and 550 ft-c ± 10 percent (1850 w/cm^2; 5920 lux according to data provided by D. P. Larsen (30) in his work with this species. Good replication has been obtained under these conditions.

** The energy level output of a bank of six 48 inch "cool white" fluorescent lamps (GE 40 watt, @ 60 Hz) was approximately 1300$\mu w/cm^2$ (range, 380-760 nm) at a distance of 26 3/4 inches, as measured with an ISCO Model SRC spectroradiometer. Using the same measurement geometry, a Weston Model 756 Illumination Meter read 400 footcandles. All reflecting surfaces were matte white.

Therefore, utilizing a calibrated illumination meter with a footcandle readout, one may, by adjusting the height of the lights, achieve a known energy level output of 1300 $\mu w/cm^2$.

For further discussion of the problems of the differences in absorption of light by photosynthesizing organisms and by man's eye and their measurement, see Tyler (41).

C. Biomass Determination

Several methods of growth measurement, indirect or direct, may be employed. Direct cell counting, using a haemocytometer with a microscope, is relatively accurate at high population levels, but very slow. Gravimetrically determined dry weights are likewise time consuming and may be at variance with calculated dry weights if substantial bacterial growth occurs in samples, or substantial amounts of ferric-magnesium hydroxide forms in the sample. Also, accurate dry weights are difficult to obtain at low algal biomass concentrations.

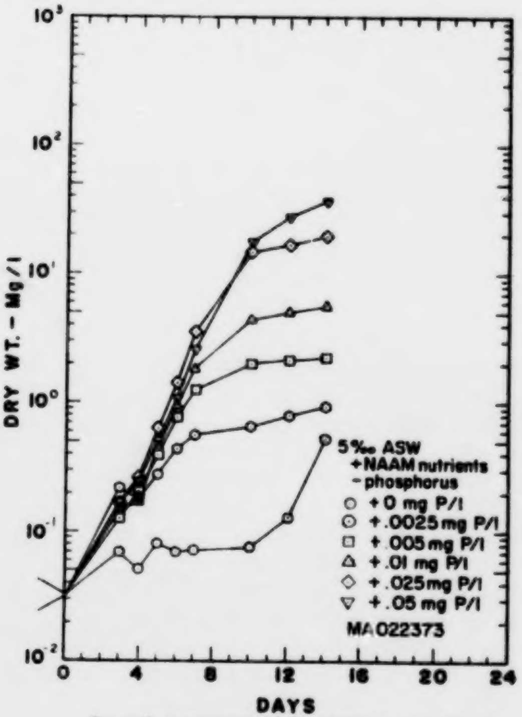
In the development of this test, electronic particle counters were used, with frequent cross checks by gravimetric dry weight determinations and haemocytometer counts. Cell counts at either 1:10 or 1:100 dilution in filtered isotonic saline electrolyte were made, recording mean cell volumes (MCV) from an accompanying mean cell volume computer. Since *Dunaliella* is substantially larger than *Selenastrum capricornutum*,aperture current and amplification settings used for *Dunaliella* are generally one-half that used to count *Selenastrum* in the freshwater assay procedure. Generally, the electronic particle counter was found to be the most feasible method of dealing with large numbers of samples, but could not be economically justified for casual or infrequent assays.

D. Nutrient Response

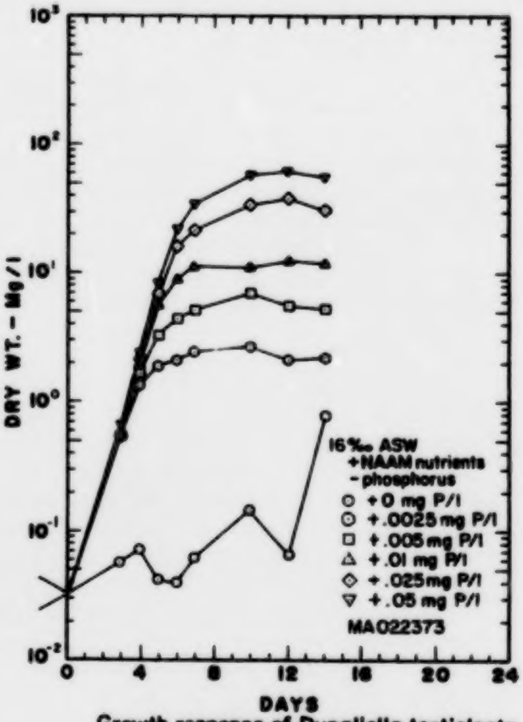
The initial assumption was that estuarine waters are nitrogen limited for algal growth (17, 21, 5, 31, 32, 33). One recent sampling survey of the Yaquina estuary showed that, while water from the lower bay was predictably nitrogen limited for algal growth, water from the upper bay and its tributaries was shown to be phosphorus limited for algal growth. The marine algal assay, using *Dunaliella,* can serve to define this boundary, which can change not only with the season but with each tide and fluctuations of tributary inflow.

With respect to this situation, the response curves developed for *Dunaliella* show that the alga will respond to concentrations at least as low as 2.5 µg P/l, 10 ug ammonia N/l, and 50 ug nitrate N/l in defined medium.

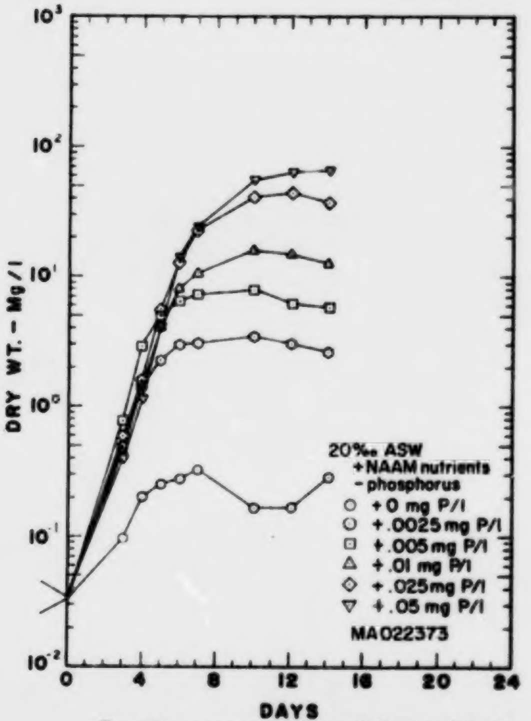
The curves were developed from additions (spikes) ranging from 2.5 to 50 µg/l in five steps for phosphorus, Figure 3; 10 to 1,000 ug in five steps for nitrate nitrogen, Figure 4 and ammonia nitrogen, Figures 5, 6. All assays



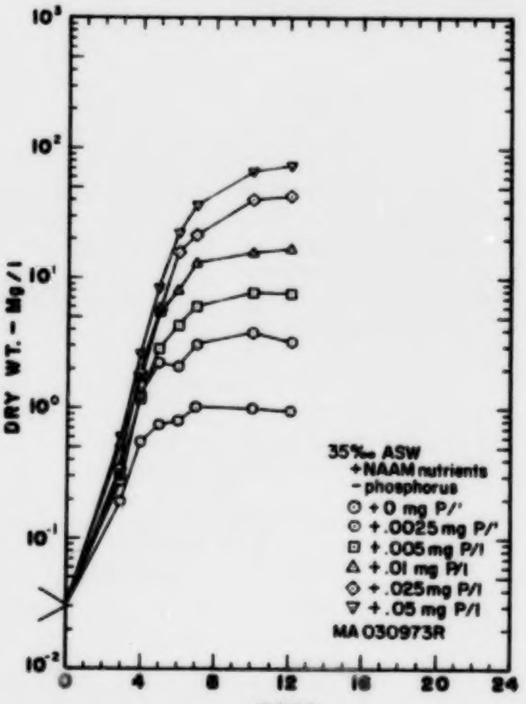
Growth response of *Dunaliella tertiolecta*



Growth response of *Dunaliella tertiolecta*

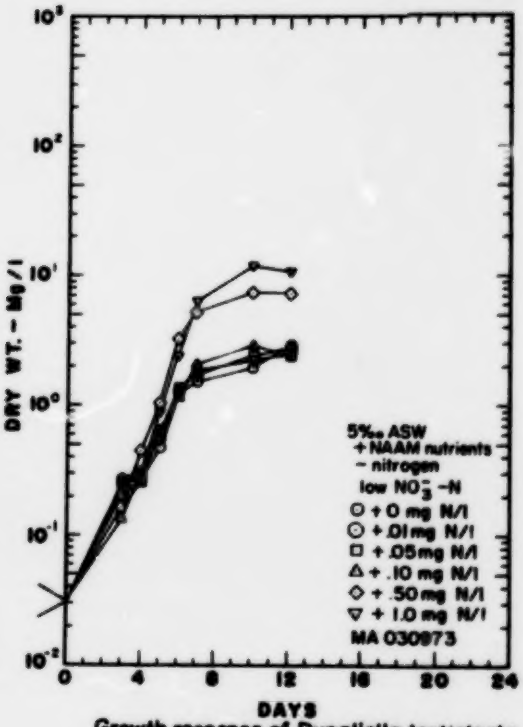


Growth response of *Dunaliella tertiolecta*

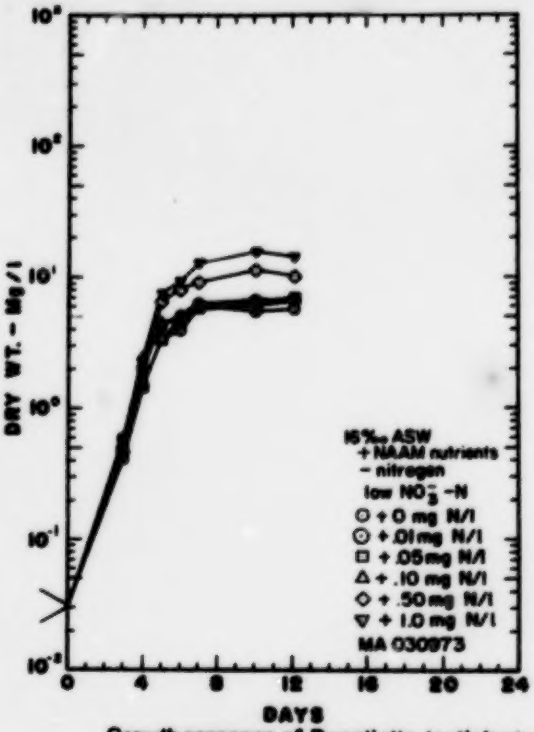


Growth response of *Dunaliella tertiolecta*

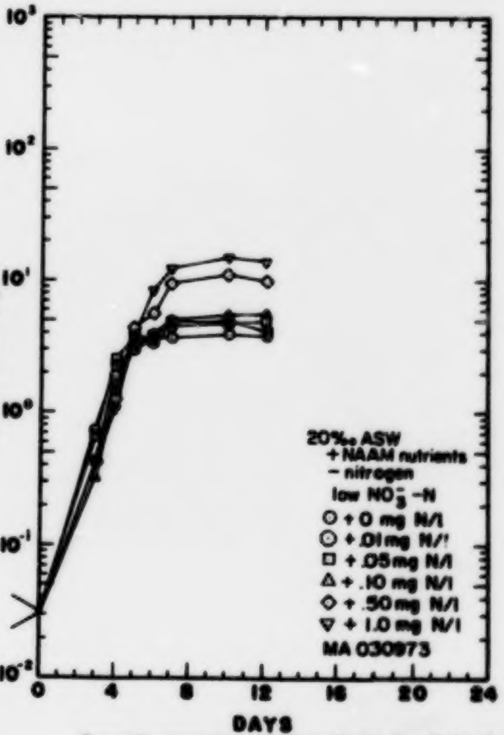
Fig 3



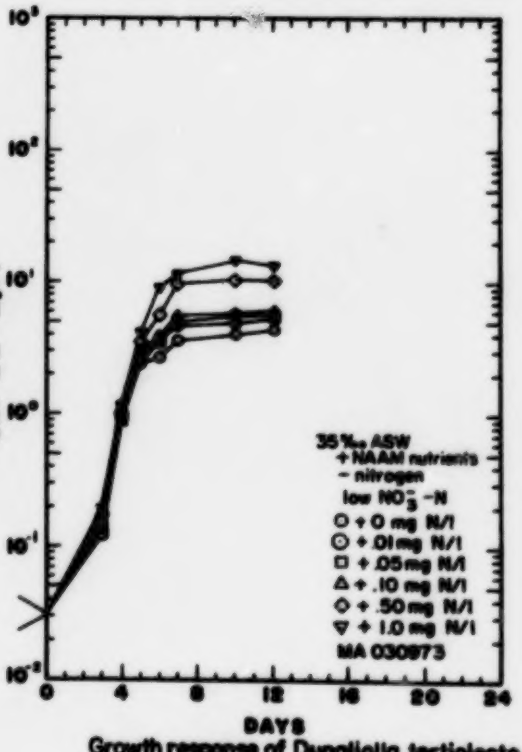
Growth response of *Dunaliella tertiolecta*



Growth response of *Dunaliella tertiolecta*

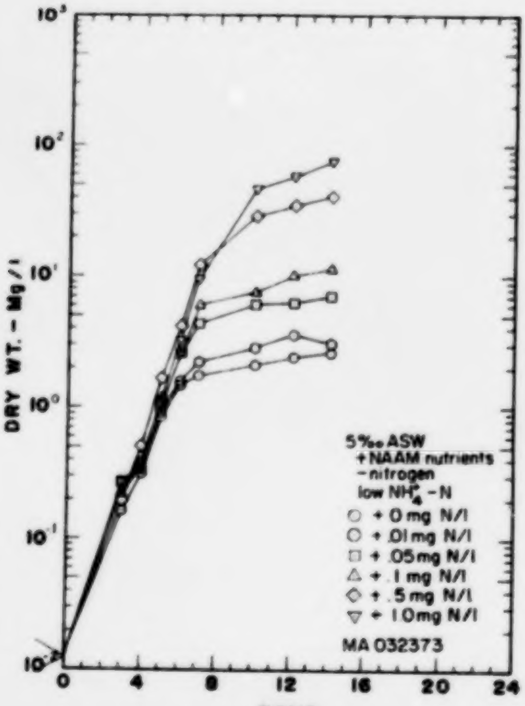


Growth response of *Dunaliella tertiolecta*

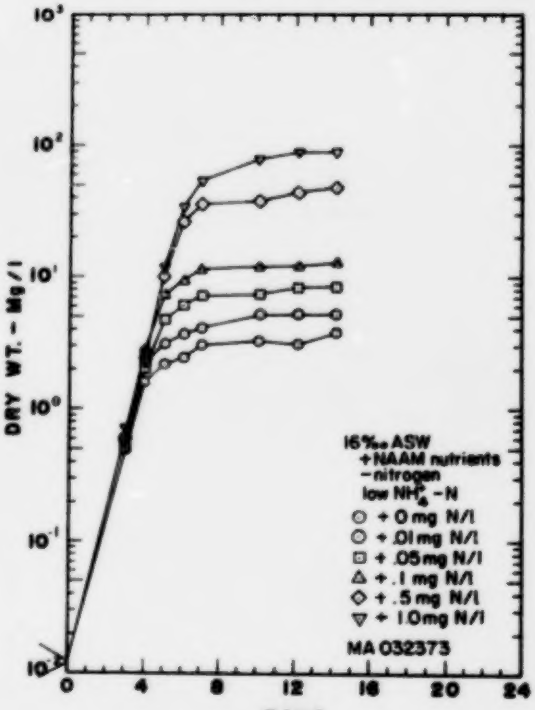


Growth response of *Dunaliella tertiolecta*

Fig. 4



Growth response of *Dunaliella tertiolect*



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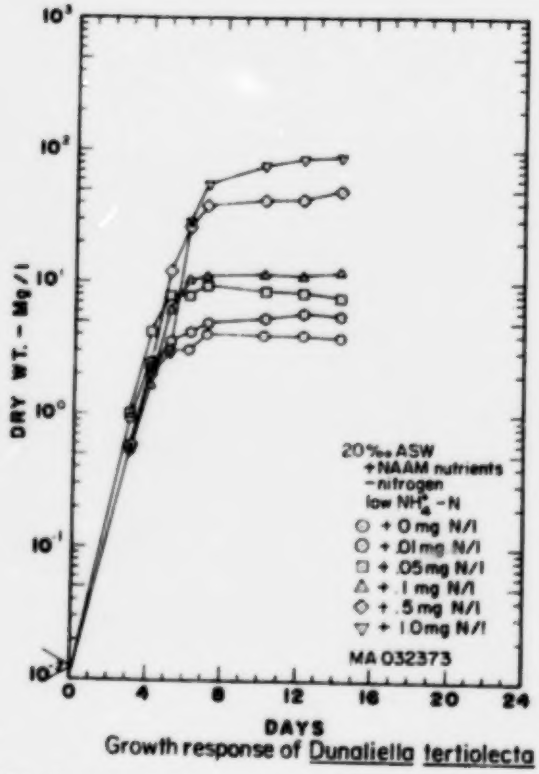
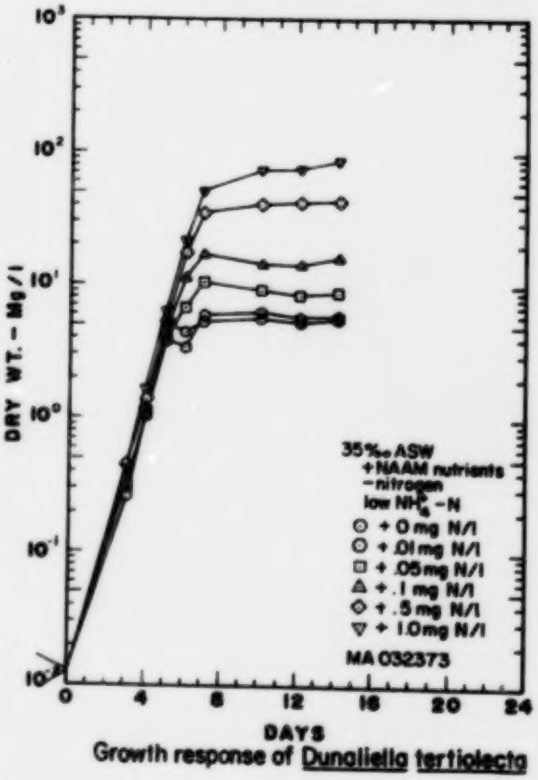


Growth response of *Dunaliella tertiolecta*



Growth response of *Dunaliella tertiolecta*

Fig. 5

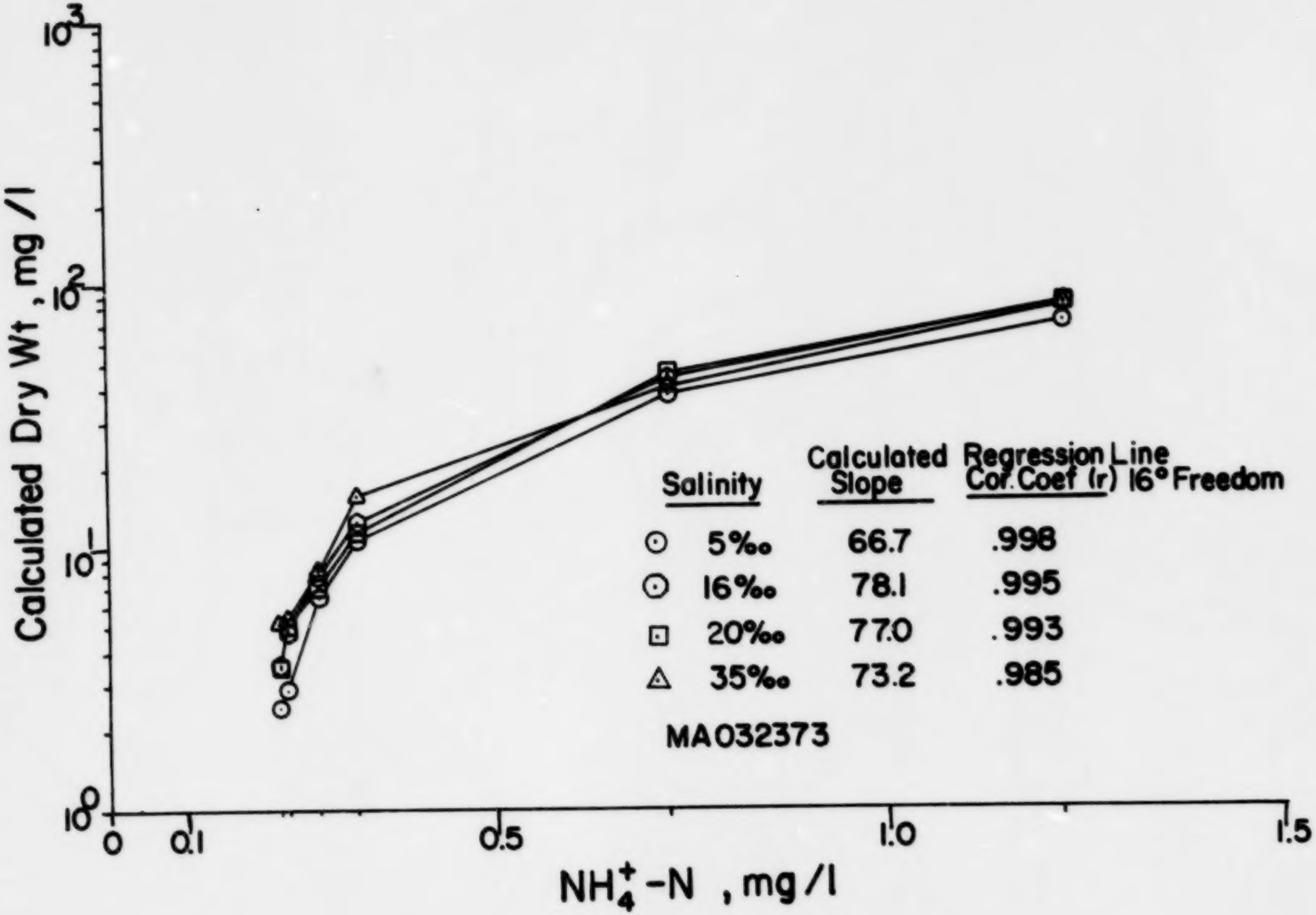


Fig. 6 Growth response of *Dunaliella tertiolecta*, day 14

were run in triplicate along with controls. Nitrilotriacetic acid (NTA) was examined as a possible nitrogen source (34, 35, 36, 11), or for possible growth depressing or enhancing effects by chelation in actual estuarine field samples. There was no significant yield difference between control and spikes ranging from 50 to 1,000 mg/l NTA nitrogen (expressed as nitrogen, 7.3 percent N by weight), Table 1.

Although ammonia nitrogen was more stimulatory than nitrate nitrogen in our ASW (37), there appeared to be a growth rate difference but no yield difference in spiked field samples, Figures 14, 15, 17 and 18.

In Burkholder's ASW (5‰ to 35‰ salinity *Dunaliella* produces an average of 1.076 mg dry weight per µg of phosphorus, 0.0318 mg dry weight per µg of nitrate nitrogen, and 0.0796 mg dry weight per µg of ammonia nitrogen, (Table 2).

E. Replication

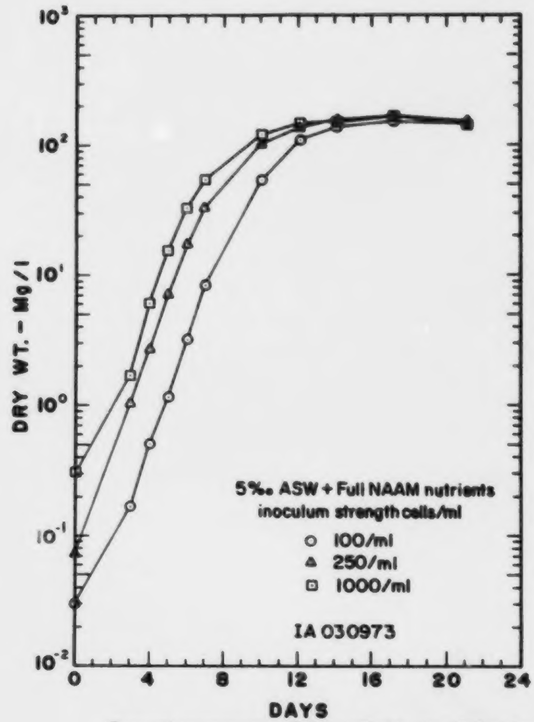
One outstanding characteristic of the use of *Dunaliella* was the consistency of data replication. An example of this is the result of the investigation of NTA as a nitrogen source in natural water samples. NTA appeared to have little, if any, effect on the growth of *Dunaliella*,either stimulatory or inhibitory. In the experiment, the control and four levels of NTA (+0.05 to 1.0 mg/l as N) with three replicates each gave a total of fifteen replicates for each of six natural waters (all Oregon coastal estuaries). In all cases, a t-test (13 degrees of freedom) showed no significant difference between control and spikes, and no correlation was shown between an increase in NTA and dry weight produced. The normalized standard deviation for the entire run of 90 flasks was less than ± 15 percent.

F. Standard Inoculum Level

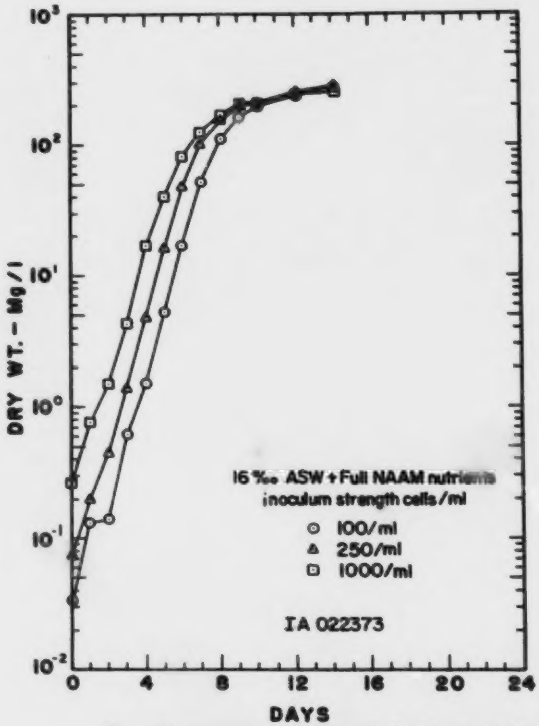
In order to avoid problems of nutrient carry-over in a healthy inoculum, the size of the inoculum was reduced from an initial 1,000 cells per ml (0.3 mg/l dry weight) to 100 cells per ml (0.03 mg/l dry weight). Although there was a detectable lag in growth of about two days, growth rate was unaffected and the final yield was virtually identical at day 10 or 12, Figure 7. The inoculum was dispensed in a 1 ml volume.

Table 2. BIOMASS PRODUCED PER UNIT OF NUTRIENT BY *DUNALIELLA* AT DAY 14 IN DEFINED MEDIA

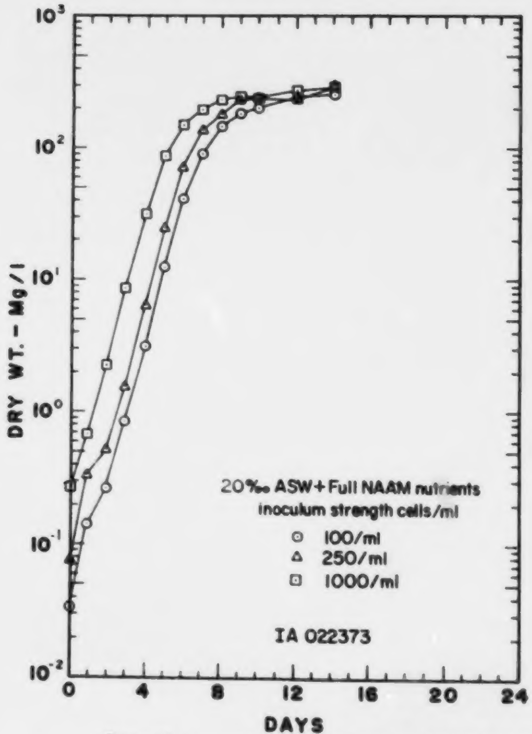
	mg dry weight/μg of nutrient				
Salinity	P	$NO_3^- - N$	$NH_4^+ - N$	$P:NO_3^- - N$	$P:NH_4^+ - N$
5 °/₀₀	0.557 ±0.158	0.0096 ±0.0014	0.0747 ±0.0075	1:58	1:7.4
16 °/₀₀	0.930 ±0.240	0.0308 ±0.0394	0.0844 ±0.0058	1:30.2	1:11.0
20 °/₀₀	1.170 ±0.164	0.0331 ±0.0379	0.0766 ±0.005	1:35.3	1:15.3
35 °/₀₀	1.129 ±0.232	0.0315 ±0.0361	0.0765 ±0.0193	1:41.1	1:13.5
Average	1.076	0.0318	0.0796	1:41.1	1:13.5



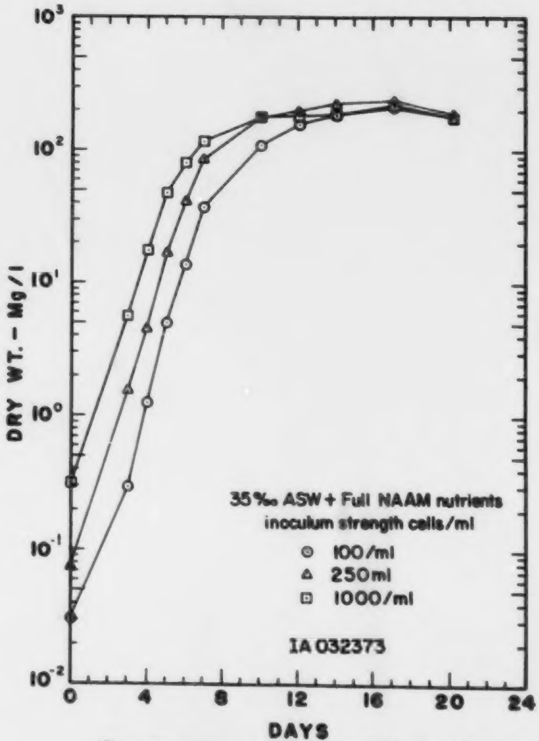
Growth response of *Dunaliella* *tertiolecta*



Growth response of *Dunaliella* *tertiolecta*



Growth response of *Dunaliella* *tertiolecta*



Growth response of *Dunaliella* *tertiolecta*

Fig 7

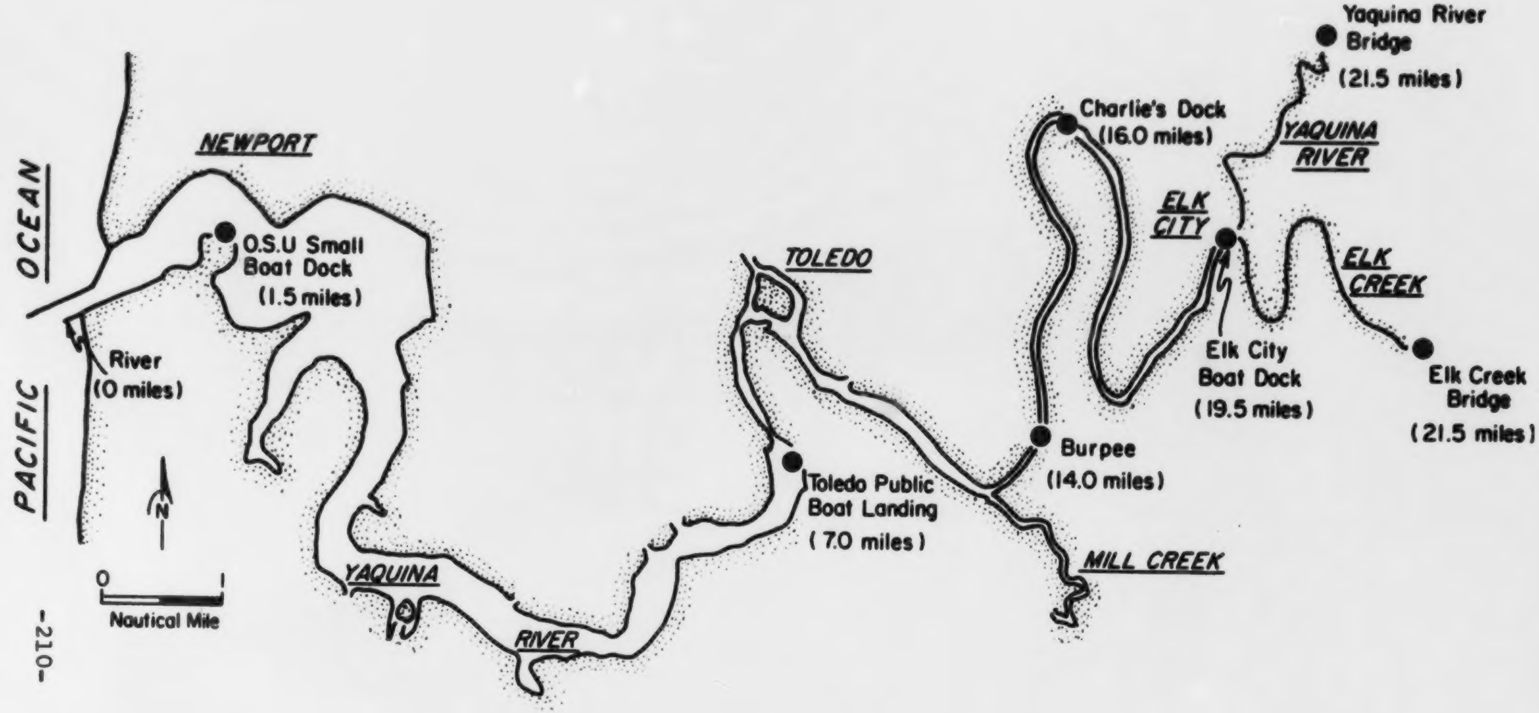
FIELD TESTS

The majority of field work has been done with water from the Yaquina Estuary, Newport, Oregon, Figure 8. It is relatively near, and has been studied extensively by other EPA and Oregon State University personnel. Some samples were also obtained from several sites in Puget Sound and assayed in cooperation with the Washington State Department of Ecology.

In general, several observations may be made: (a) the marine algal assay indicated whether nitrogen or phosphorus constituted a growth-limiting nutrient in estuarine waters; (b) with a somewhat extended incubation period for bioassay, the test organism could adjust to the lower salinities of freshwater tributaries [although a lower limit of 3.5 ‰ salinity has been noted for Dunaliella (25)] to indicate whether nitrogen or phosphorus was algal growth limiting; and (c) the bioassay could show the point of changeover in the estuary from phosphorus to nitrogen limitation.

Because of the likelihood of growth limitation by light or elements other than nitrogen or phosphorus (iron, manganese, or, in the case of diatoms, silicon) this bioassay may not predict the full potential of a water to produce algal growth. However, it does seem reasonable that the indications of nutrient limitation given by the assay should be accurate in a relative sense (38). It should be kept in mind all samples were membrane filtered and stored at 4°C until assayed.

The ability of Dunaliella to respond to low level nitrogen spikes to a natural water sample can be seen in Figure 9, which illustrates the changeover from nitrogen to phosphorus limitation in a sample from the upper Yaquina estuary. In this case, in addition to control, nitrate nitrogen was added at the levels of 0.02, 0.1, 0.2, 0.5, and 1.0 mg/l as nitrogen (0.001 to 0.05 mg P/l, and N + P combinations were also added). The growth response started to decline just below 0.5 mg/l total nitrogen, plateauing at about 23 mg dry wt/l with the 1.0 mg N/l spike. However, if 0.05 mgP/l was added in addition to the 1.0 mgN/l spike, approximately 36 mg dry wt/l was attained. If all points on the plot are considered, except the 1.0 mg N/l set, a line of very good fit can be plotted ($r = .986$, $t = 26.7$, 19 degrees of freedom, significant at the 0.1 percent level) showing that about 97 percent of the change in dry weight can be attributed to a change in the level of nitrogen present. If one considers only nitrogen, the plateau of 23 mg dry weight/l defined by the 0.5 and 1.0 mg N/l spikes


OCEAN
PACIFIC
NEWPORT
O.S.U Small
Boat Dock
(1.5 miles)
River
(0 miles)
N
0
1
Nautical Mile
YAQUINA
RIVER
TOLEDO
Toledo Public
Boat Landing
(7.0 miles)
Burpee
(14.0 miles)
MILL CREEK
Charlie's Dock
(16.0 miles)
ELK
CITY
Elk City
Boat Dock
(19.5 miles)
YAQUINA
RIVER
Yaquina River
Bridge
(21.5 miles)
ELK
CREEK
Elk Creek
Bridge
(21.5 miles)

MARINE ALGAL ASSAY FIELD SAMPLING SITES
YAQUINA ESTUARY, NEWPORT, OREGON

Fig. 8

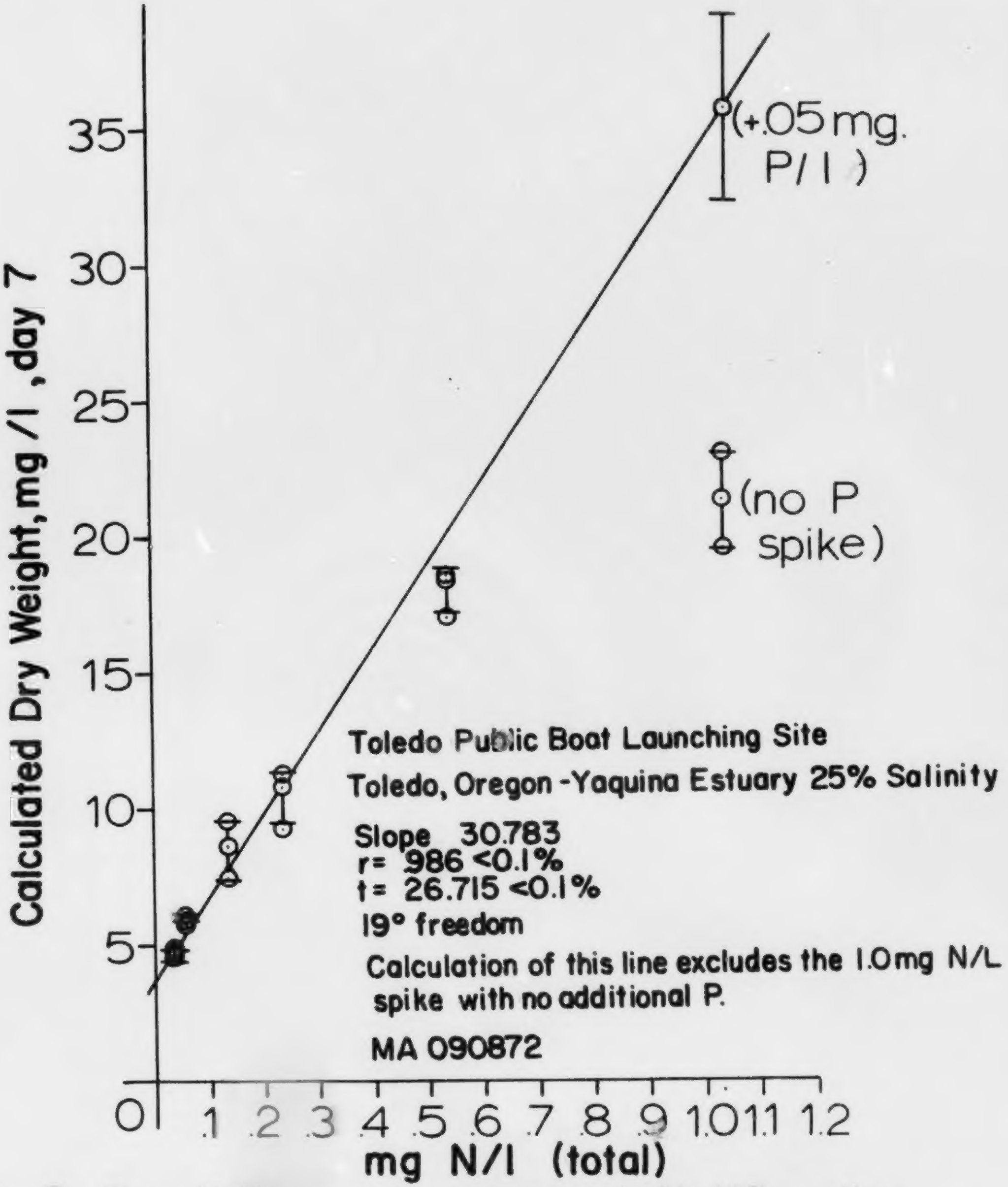


Fig.9 Dunaliella growth response to $NaNO_3$ spikes
Plot shows advent of phosphorus limitation, given enough nitrogen in nitrogen limited water

Table 3. CHEMICAL PARAMETERS OF YAQUINA ESTUARY AND TRIBUTARY SURFACE GRAB SAMPLES, 28 **June** 1973, AT OR NEAR HIGH WATER, ON INCOMING TIDE

Sampling Site Parameter	OSU Small Boat Dock	Toledo Public Boat Landing	Burpee	Charlie's Dock	Elk City	Elk River, Upstream	Yaquina River, Upstream
NH_4^+ -N- mg/l	0.036	0.018	0.076	0.06	0.038	0.025	<0.001
NO_3^- -N- mg/l	0.120	0.129	0.416	0.430	0.498	0.315	0.560
Tot. sol. P mg/l	0.035	0.015	0.015	0.015	0.015	0.015	0.01
Ortho-P mg/l	0.03	0.013	0.011	0.019	0.009	0.011	0.008
Sulfate, mg/l	3100	896	256	10	3	<2.5	4
Sol. Fe, µg/l	240	100	100	140	140	160	140
Sol. Mn, µg/l	20	27	34	11	13	10	9
Salinity, $^0/oo$	30.76	14.14	2.882	<2.84	<2.84	<2.84	<2.84
Conductivity, µmho	49,200	23,200	5,800	208	69	67	65
Tot. Sol. C, mg/l	24.5	15.8	9	5.4	4.7	4.8	4.5
Sol. reactive Si, mg/l	.68	2.2	4.6	4.8	5.25	5.4	5.5

demonstrates the advent of phosphorus limitation in this sample. The multiple levels of spikes also show that, when nitrogen is limiting, growth responses are linear with respect to the spike level until some other nutrient or physical factor becomes limiting.

Control dry weights for a series of bioassays by the Washington State Department of Ecology on Puget Sound samples collected by EPA Region X personnel are shown in figure 10. A plot of day 10 dry weights against total nitrogen accounted for 83 percent of the variation in dry weight, indicating that nitrogen was probably the limiting nutrient for algal growth.

Phosphorus was found to be limiting in a sample taken on November 1, 1972 at the Burpee sampling site one day after the onset of heavy winter rains, Figures 11, 12. Nitrogen, however, appeared to be limiting downstream at the Toledo and OSU dock stations. After 6 weeks of sample storage, the status of the Toledo sample as probably nitrogen limiting was confirmed and the phosphorus limitation in the Burpee sample was reaffirmed. This indicated that the apparent phosphorus limitation was real and not a condition related to decreased salinity. Instead it was probably caused by the increased nitrogen content of the rainwater runoff.

Figure 13 shows the results of assays on water samples collected at the same site on August 1972. It can be seen from control flasks that growth correlates very well with total N but not with salinity. On June 28, 1973 samples were taken in the Yaquina estuary, Figure 14, from OSU dock, Elk City, and approximately 2 miles further up each of the two tributaries, Elk River and Yaquina River. Salinities ranged from 30.8‰ (49,300 μmho conductivity) at the OSU boat dock station to less than 2.8‰ (65 μmho) in the Yaquina River station [See Table 3 for chemical analysis of sample (40)].

After physiological adjustment, Dunaliella grew in these essentially freshwater situations and gave a statistically significant indication of the growth limiting nutrient, Figures 14, 15. In this case, it is evident from the curves that nitrogen was limiting at the OSU dock station, Figure 14. At the Toledo station (14‰ salinity) the situation is somewhat indistinct; however a t-test shows that the growth in the sample spiked with phosphorus is significantly greater at the 5 percent level than that in the control or the nitrate and ammonia spiked samples. These tests indicate phosphorus limitation.

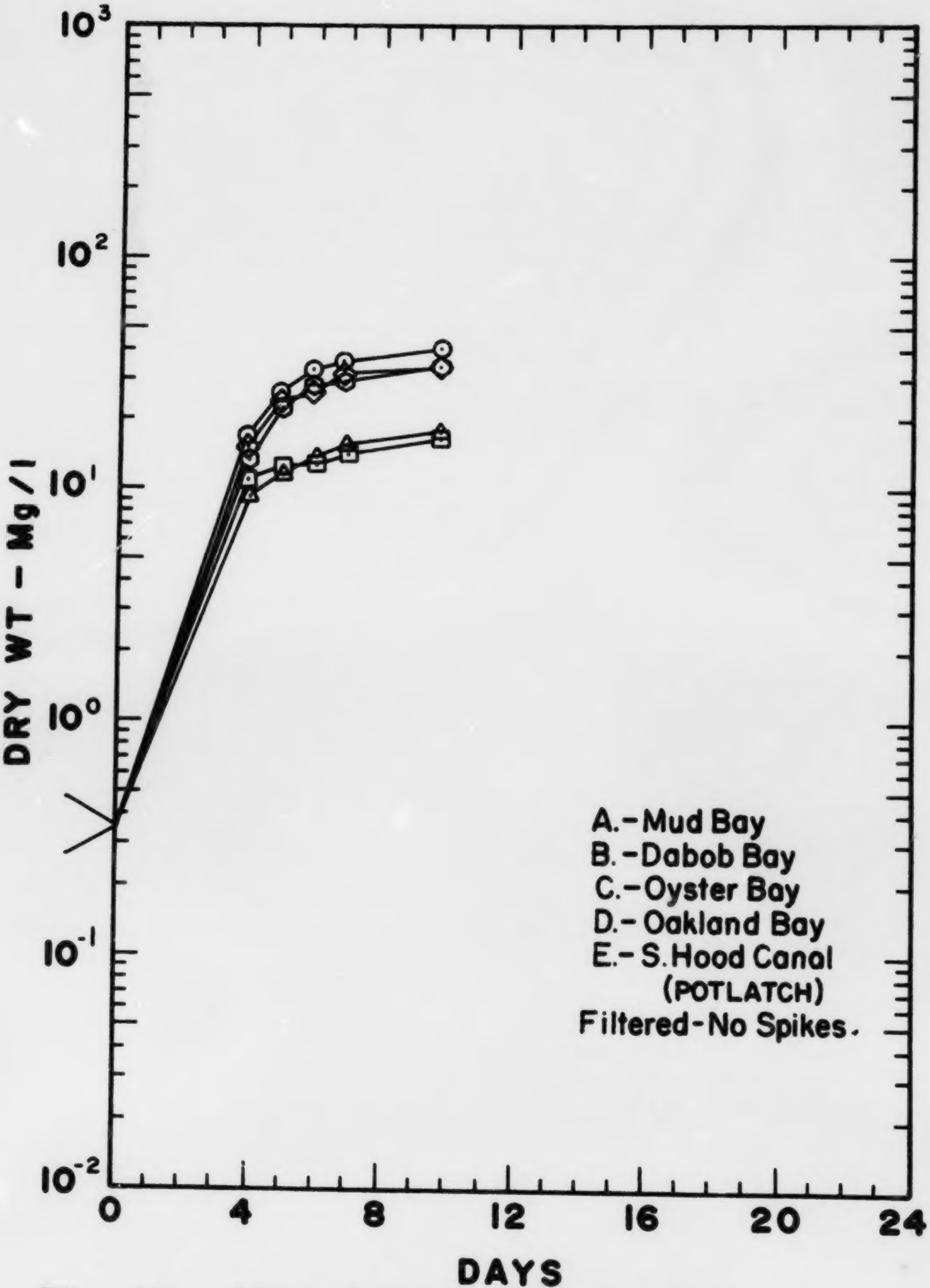
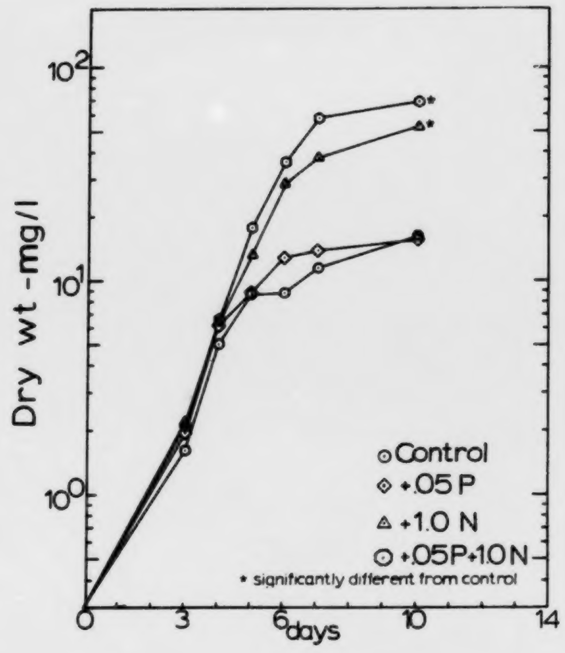
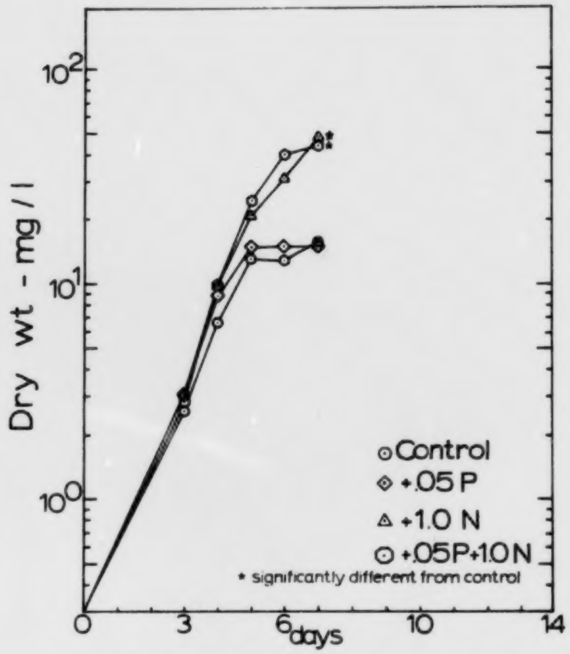


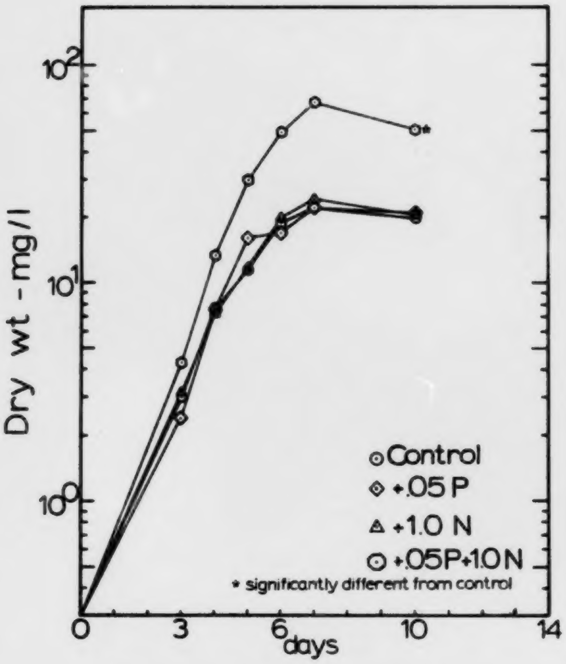
Fig 10 Algal Assay of Puget Sound Sites Spring 1973



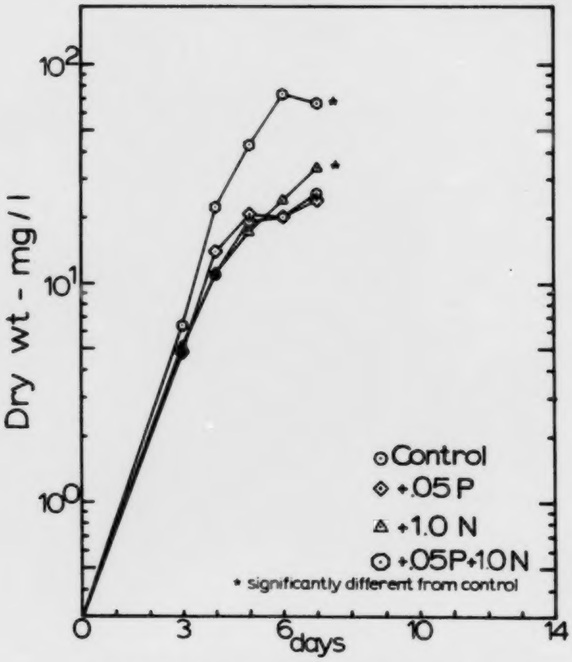
Growth response of *Dunaliella tertiolecta*
Yaquina Bay, OSU Dock, 11/1/72 33.8‰ salinity



Growth response of *Dunaliella tertiolecta*
Yaquina Bay, OSU Dock, 11/1/72 33.8‰ salinity
(sample stored 6 weeks)

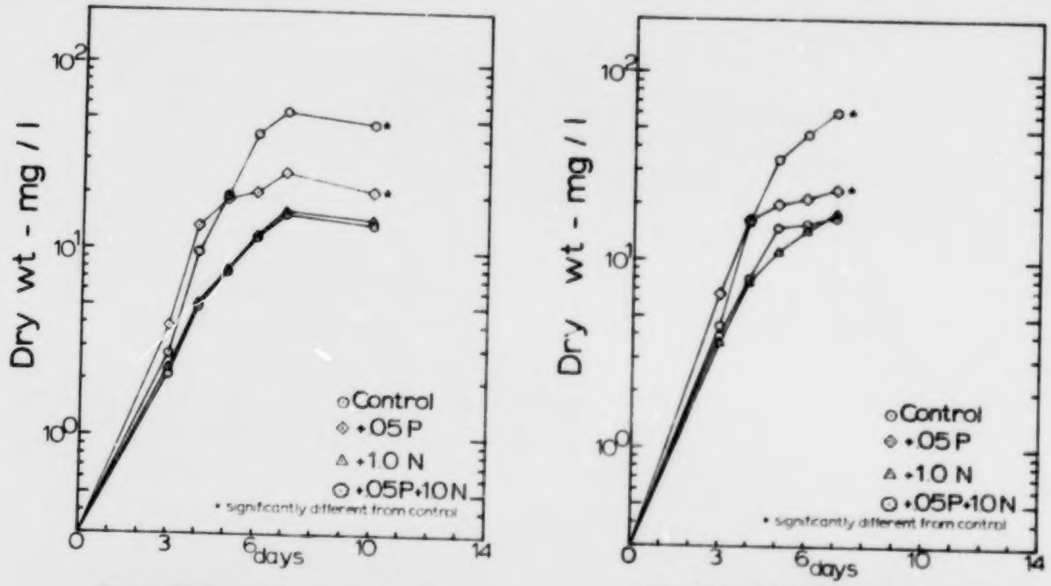


Growth response of *Dunaliella tertiolecta*
Yaquina Bay, Toledo, 11/1/72 24‰ salinity



Growth response of *Dunaliella tertiolecta*
Yaquina Bay, Toledo, 11/1/72 24‰ salinity
(sample stored 6 weeks

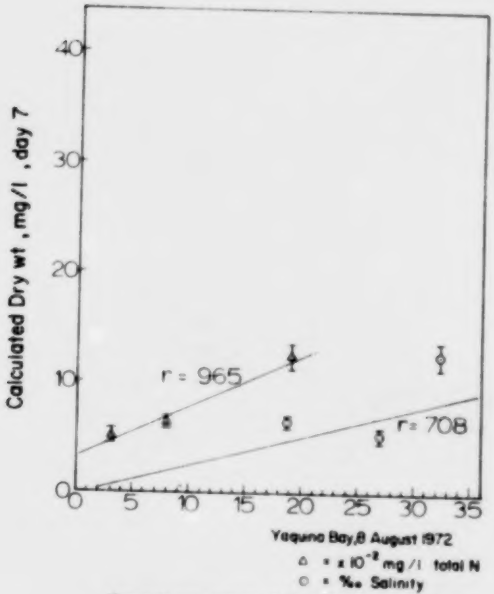
Fig. 11



Growth response of *Dunaliella tertiolecta*
Yaquina Bay, Burpee, 11/1/72, 17‰ salinity

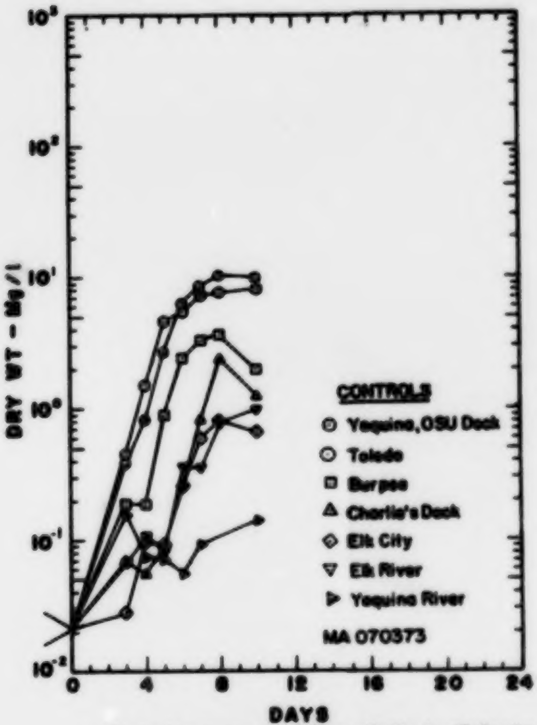
Growth response of *Dunaliella tertiolecta*
Yaquina Bay, Burpee, 11/1/72 17‰ salinity
(sample stored 6 weeks)

Fig 12

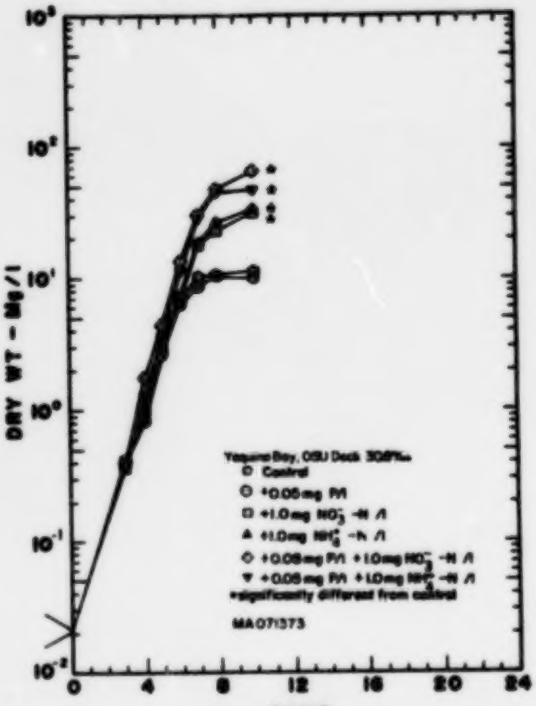


Growth response of *Dunaliella tertiolecta*

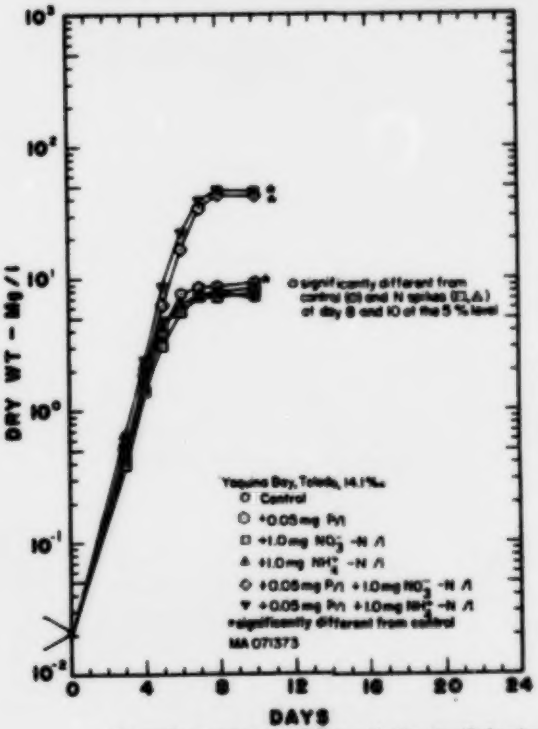
Fig 13



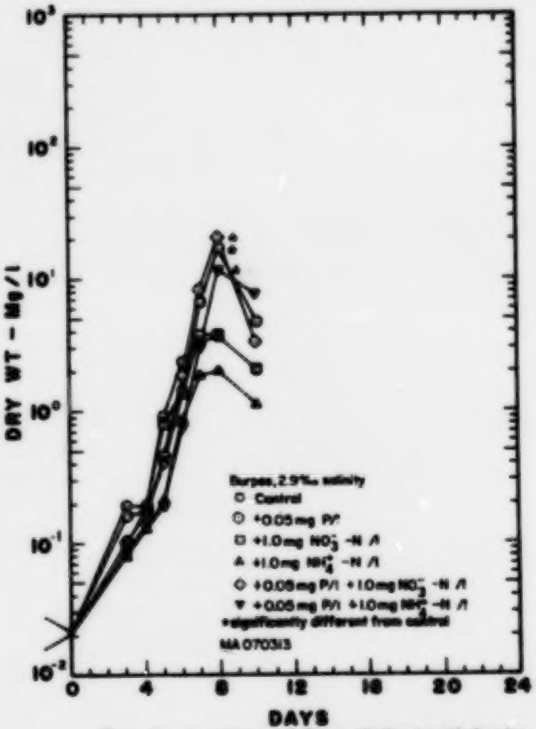
Growth response of *Dunaliella* *tertiolecta*



Growth response of *Dunaliella* *tertiolecta*

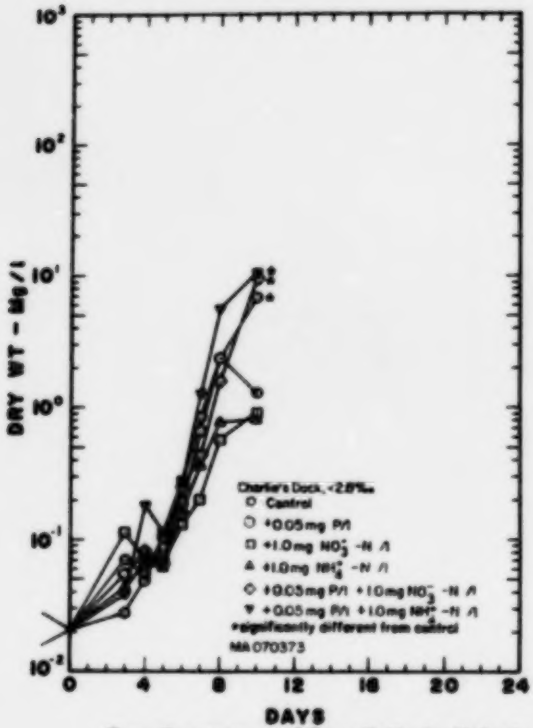


Growth response of *Dunaliella* *tertiolecta*

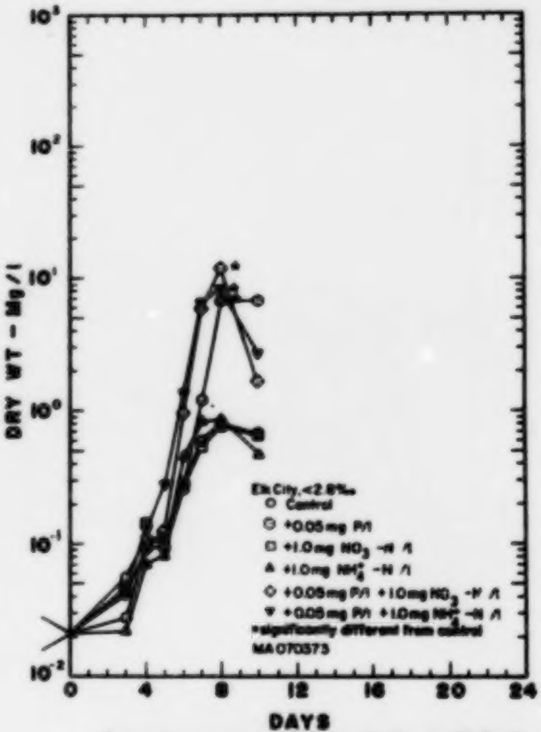


Growth response of *Dunaliella* *tertiolecta*

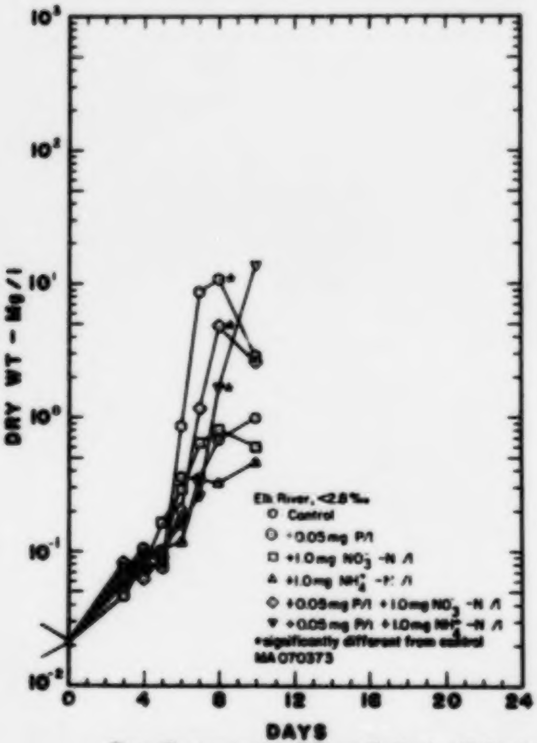
Fig. 14



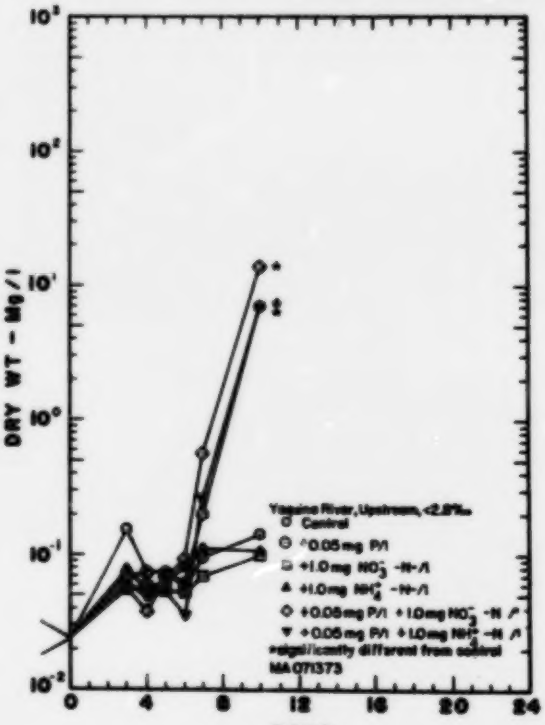
Growth response of *Dunaliella tertiolecta*



Growth response of *Dunaliella tertiolecta*

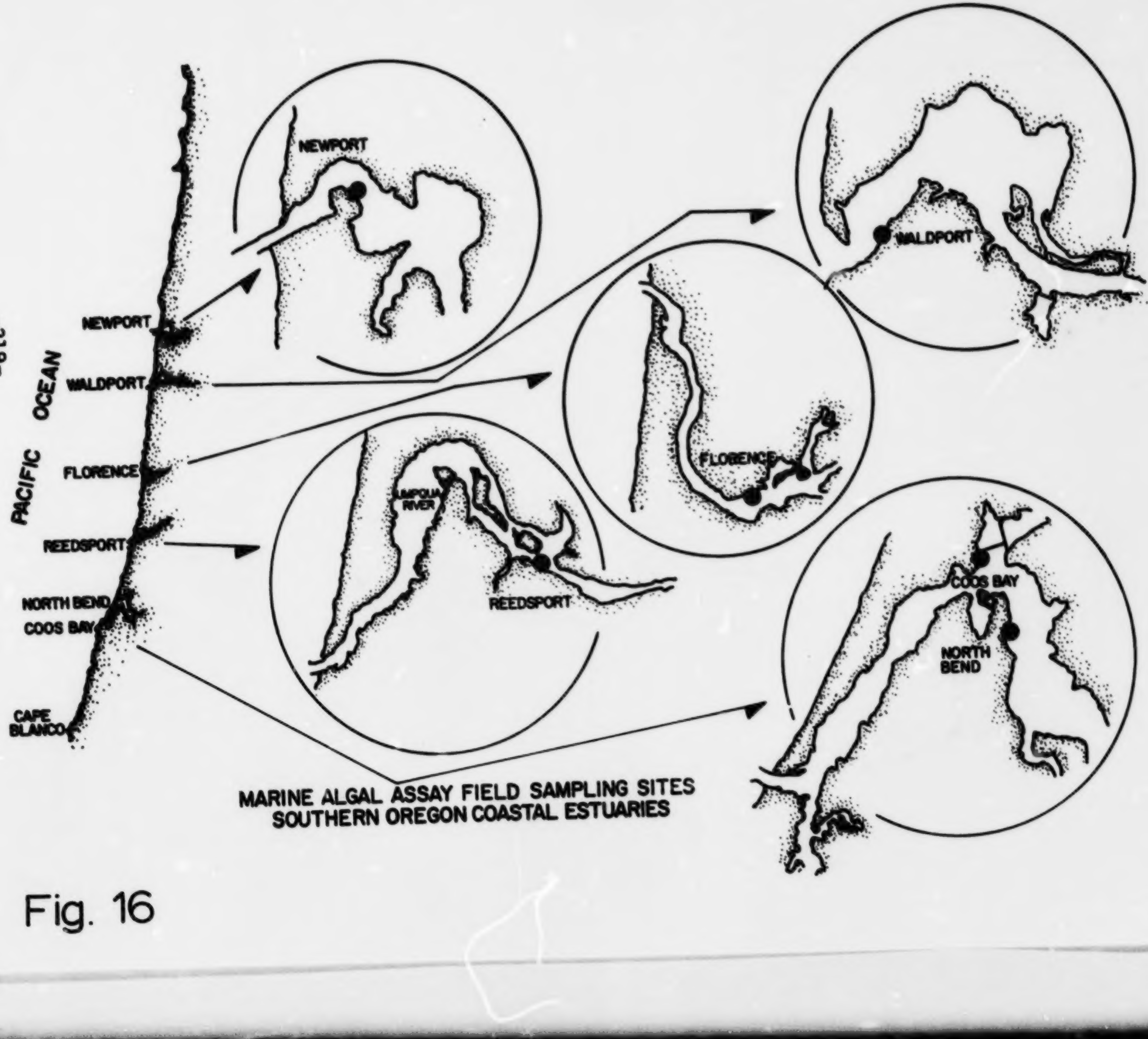


Growth response of *Dunaliella tertiolecta*



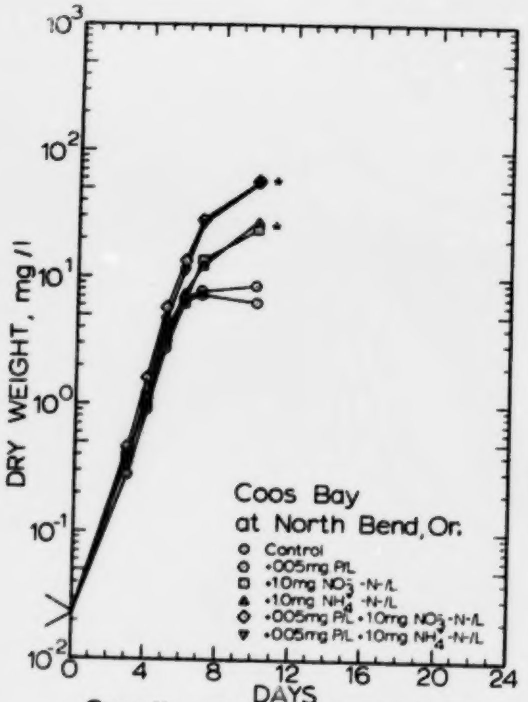
Growth response of *Dunaliella tertiolecta*

Fig 15

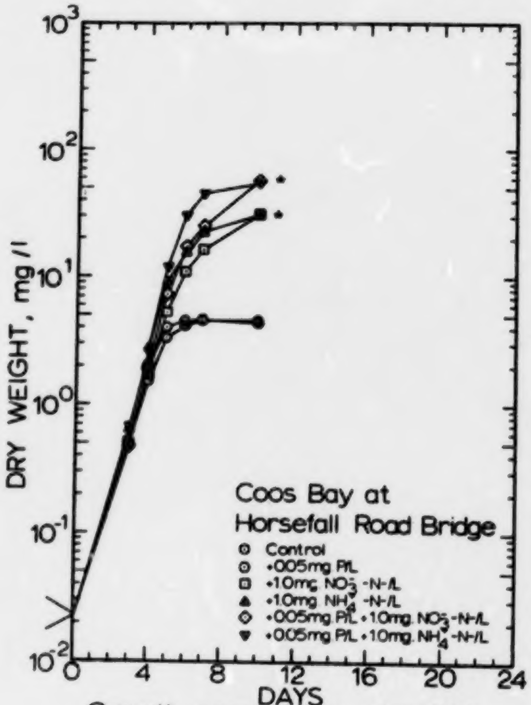

NEWPORT
WALDPORT
FLORENCE
REEDSPORT
NORTH BEND
COOS BAY
CAPE BLANCO
PACIFIC OCEAN
NEWPORT
WALDPORT
FLORENCE
UMPQUA RIVER
REEDSPORT
COOS BAY
NORTH BEND

MARINE ALGAL ASSAY FIELD SAMPLING SITES
SOUTHERN OREGON COASTAL ESTUARIES

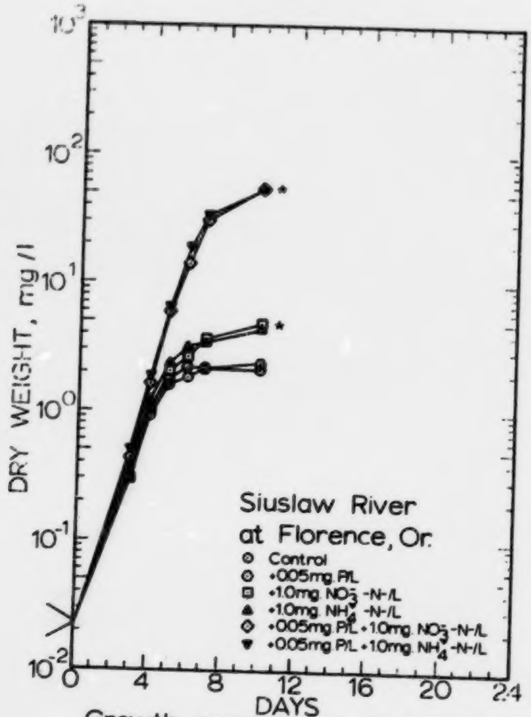
Fig. 16



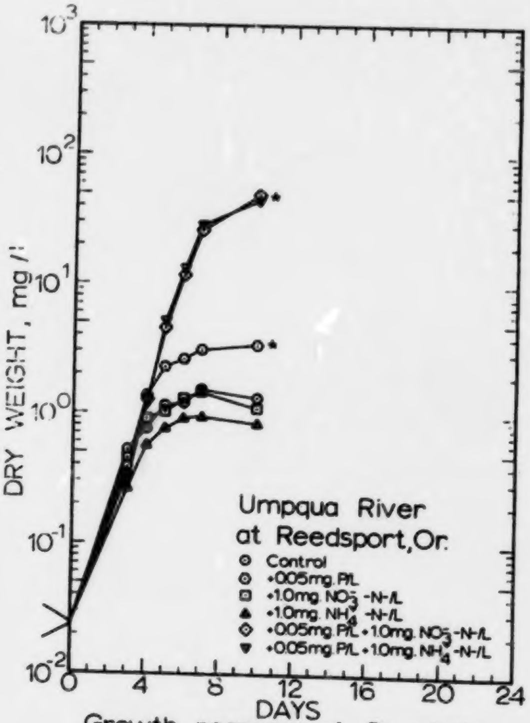
Growth response of *Dunaliella tertiolecta*



Growth response of *Dunaliella tertiolecta*

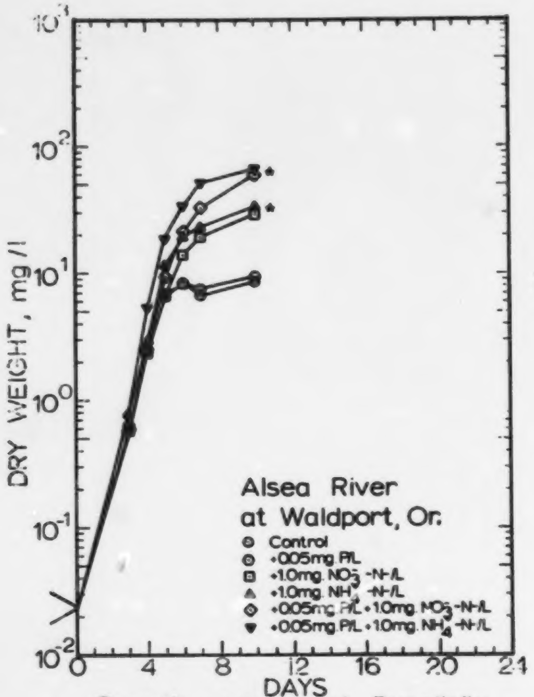


Growth response of *Dunaliella tertiolecta*

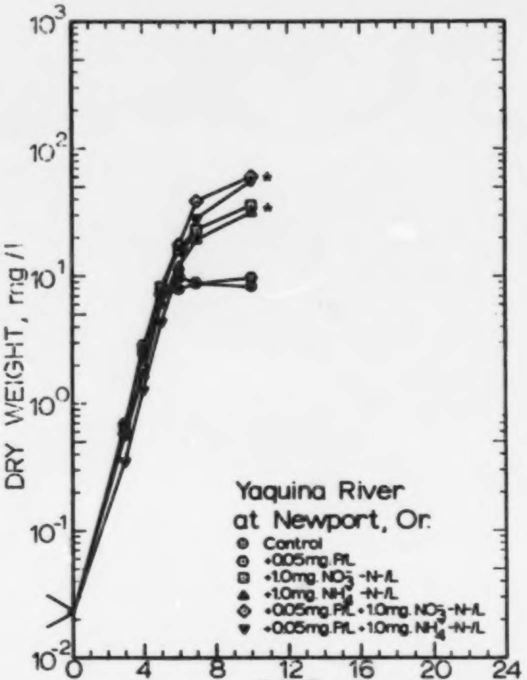


Growth response of *Dunaliella tertiolecta*

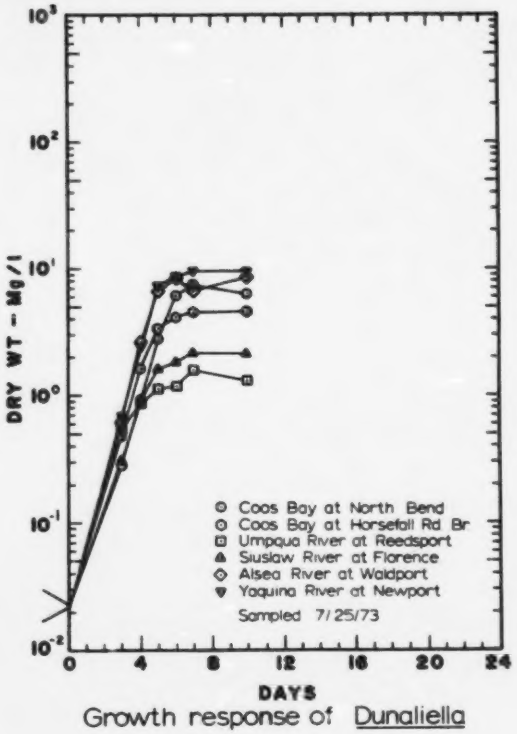
Fig. 17



Growth response of Dunaliella tertiolecta



Growth response of Dunaliella tertiolecta



Growth response of Dunaliella

Fig. 18

Table 4. CHEMICAL PARAMETERS OF 6 OREGON ESTUARY SAMPLES AFTER MEMBRANE FILTRATION
Surface Grab Samples, 25 July 1973,
on Incoming Tide at or near High Water

Sampling Site Parameter	Coos Bay at North Bend	Coos Bay at Horsefall Rd. Bridge	Umpqua River at Reedsport	Siuslaw River at Florence	Alsea River at Waldport	Yaquina River at Newport
NH_4^+ -N- mg/l	0.145	0.118	0.027	0.059	0.103	0.100
NO_3^- -N- mg/l	0.044	0.019	0.003	0.009	0.082	0.11
Total P mg/l	0.03	0.035	0.01	0.01	0.035	0.035
Ortho-P mg/l	0.028	0.028	<0.001	0.01	0.036	0.03
Sulfate mg/l	3200	3300	1500	2500	3400	3700
Sol. Fe, μg/l	240	240	80	120	240	240
Sol. Mn, μg/l	40	40	20	20	30	30
Salinity, $^o/_{oo}$	31.18	31.14	11.62	21.97	33.09	32.88
Conductivity, μmho	47,500	47,500	19,500	35,000	50,400	50,300
Tot. Sol. C, mg/l	25	26	13	18	25	25
Sol. reactive Si, mg/l	0.4	0.4	2.5	0.72	0.84	0.72

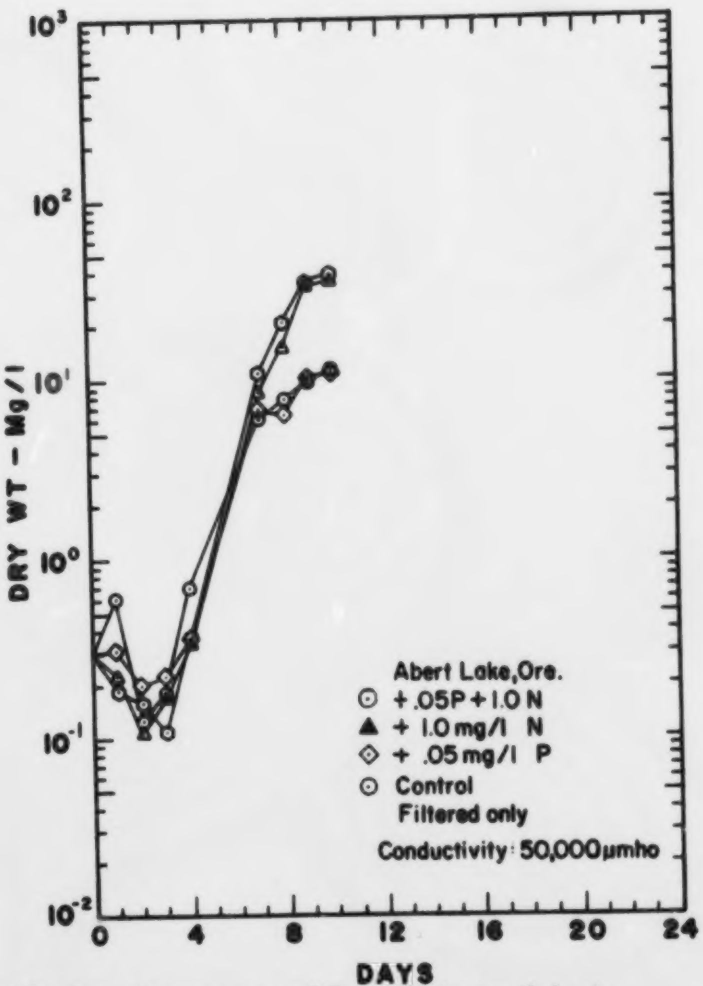
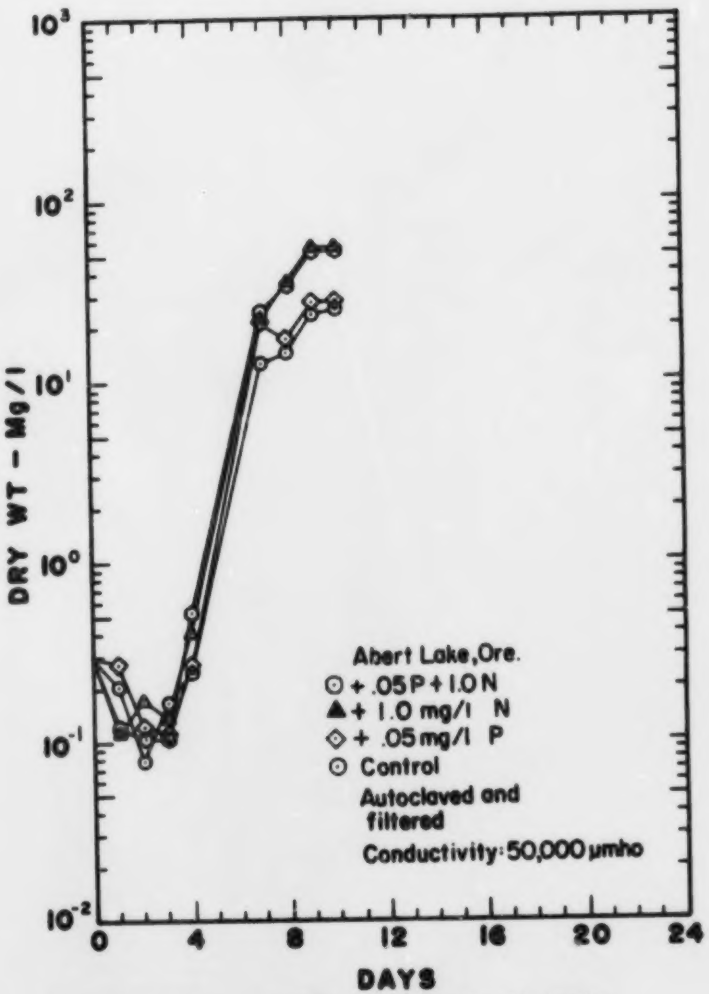


Fig.19 Growth response of Dunaliella tertiolecta in alkaline fresh water



Growth response of Dunaliella tertiolecta in alkaline fresh water

At the Burpee station, Figure 14, (2.9 ‰ salinity, 5800 μmho) phosphorus was shown to be the limiting growth factor. Sample spiked with phosphorus grew significantly more than control or those spiked with nitrogen.

Samples from the remainder of the stations, all essentially freshwater (208 -65 μmho conductivity), showed marked lags in growth patterns. All of these stations responded significantly to spikes containing phosphorus but not nitrogen, which were not significantly different than control. It should be noted that replication was not as good in low salinity or freshwater as in higher salinities with *Dunaliella*, but more experimentation should yield a workable test protocol to establish acceptable replicability.

The next set of samples was taken on July 25, 1973, from five southern Oregon coastal estuaries, from Coos Bay on the south to Yaquina Bay on the north (Figure 16). These estuaries, sampled on the incoming tide at or near high water, were nitrogen limited with the exception of the Umpqua River at Reedsport. This sample, 11.6 ‰ salinity, responded in a significant manner to spikes containing phosphorus, but not to nitrogen [Figures 17, 18; See Table 4 for chemical analysis of samples (40)].

A further application of the marine assay using D. *tertiolecta* is exemplified by results obtained on water samples from Albert Lake, a highly alkaline (50,000 μmho) drainage sink in inland southeastern Oregon. Attempts to assay this water with *Selenastrum capricornutum* using the freshwater Algal Assay Procedure failed when the inoculum was killed by the high alkalinity (salinity). However, from Figure 19 we can see that after an initial adjustment lag, *Dunaliella* shows nitrogen to be the growth limiting nutrient.

VI. SUMMARY

The effort to design and evaluate a marine version of the Algal Assay Procedure: Bottle Test has resulted in the establishment of the suitability of *Dunaliella tertiolecta* Butcher (DUN clone) as a highly versatile and consistent bioassay organism for nutrient assessment in marine, estuarine, and some freshwater situations.

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MONITORING SEAWATER FOR RADIONUCLIDES

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Introduction

Radioactivity has been monitored in the environment to assess radiation exposure and protect public health for approximately thirty years. The first source of radiation pollution was atomic energy research laboratories, then fallout from testing nuclear weapons was added, and most recently effluents from nuclear power stations and associated facilities have become a primary concern. The world-wide fallout from nuclear test explosions in the atmosphere stimulated extensive measurements and research to describe the movement of radionuclides in the environment, including the marine ecosystem. Some of the attempts to interpret marine radioactivity data led to programs for studying transport phenomena with artificial and natural radionuclides as tracers.

Measurements of radionuclides in seawater by many nations are thoroughly documented. The state of the art and current trends are indicated by proceedings of a symposium sponsored by the International Atomic Energy Agency (IAEA) at Seattle in 1972 (1), and in a recent comprehensive review by the NAS-NRC Panel on Radioactivity in the Marine Environment (2). Monitoring methods and problems are summarized in two IAEA publications on marine radioactivity studies (3, 4); the more recent one (3) has probably the best available discussion of problems and possible solutions.

The basis for monitoring radioactivity is the consideration of critical radionuclides, pathways, and populations (5), where "critical" is defined as most important in leading to radiation exposure. This approach is necessary because there are too many radionuclides, available pathways, and potentially exposed persons to

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consider all with equal effort. Computational models of radiation exposure may be developed to identify and quantify the critical components, with research undertaken to evaluate uncertain vectors and measure dispersion and transfer factors. A primary purpose of a monitoring program is to test compliance with legal limits; monitoring data may also be used to confirm computational models, provide numerical factors applicable to the specific locale and check source terms.

The main considerations that shape programs for monitoring radioactivity in the marine environment are:

1. numerous radionuclides have to be measured;
2. radionuclides in seawater are at extremely low concentrations--levels of the order of 0.1 to 1 picocurie (pCi) per liter, that correspond in the case of ^{90}Sr, for example, to approximately 10^{-17} M;
3. some radionuclides take several chemical or physical forms unlike those of indigenous stable nuclides; and
4. potential radiological hazards usually arise from concentration of radionuclides in biota exposed to seawater.

Analytical Procedures

Radiochemical analysis of seawater usually combines radionuclide accumulation, chemical separation, and nuclear radiation detection. Whether all three are needed depends on the nuclear and chemical characteristics of the radionuclide, its concentration relative to the required measurement sensitivity, and possible interferences. For example, radionuclides that emit relatively penetrating gamma rays and are measured only to assure compliance with radiation protection standards (6) may not have to be concentrated or separated chemically.

Distinctly different approaches are followed if the analysis requires a few milliliters, a few liters, or hundreds to thousands of liters. In the first case, the sample is treated like other liquids (7, 8) by radioanalytical procedures such as the ones compiled by the NAS-NRC Radiochemistry Subcommittee (9). In the second case, these methods are adapted, with some modifications, to concentrate the radionuclide from the initially larger volumes of water. In the third case, very large volumes of seawater usually are passed through collectors that have been found effective for one or several radionuclides of interest and the accumulated radionuclides are then analyzed. As an alternative to these analyses, the radionuclide concentration may be measured in situ by suspending a radiation detector over or within the water, or exposing a measured fraction of the water to the detector.

Concentration of radionuclides from water has been accomplished by coprecipitation, ion exchange, absorption or solvent extraction. Many of the processes for simultaneously concentrating a variety of radionuclides are not applicable for seawater, however, because of its high salt content. A complete review of all concentration methods used for determining radionuclides from seawater would be prohibitively long, but some of the concentration techniques employed for the more radiologically important or frequently analyzed radionuclides are listed in Table 1. Concentration by coprecipitation with $Fe(OH)_3$ or absorption on MnO_2 has been most widely used. Among the radionuclides included, ^{90}Sr and ^{137}Cs have been been of primary concern because of their accumulation in seawater from global fallout. Most procedures for ^{90}Sr involve precipitation of strontium carbonate or oxalate. Cesium-137 is generally concentrated by absorption on a variety of inorganic ion exchangers.

Although the concentration methods listed in Table 1 have been used for volumes up to 1000 liters, their application for sample volumes in excess of several hundred liters is limited by need for special equipment or facilities for handling large volumes. A sampler developed by Silker et al. (32) for concentrating radionuclides from seawater eliminated this limitation. The sampler combines filtration of particulate radionuclides and absorption of dissolved radionuclides on an aluminum oxide bed in an integral unit. Where several thousand liters must be processed, seawater is pumped through the sampler at a flow rate of 40 liters/minute. The main disadvantage of the system is the low retention efficiency for many radionuclides on the aluminum oxide bed. For monitoring purposes, the collection efficiency for the radionuclides of interest must be determined.

Recent studies (33) at a nuclear power station that discharges liquid wastes into seawater agree with earlier observations (1) that effluent radionuclides are not necessarily in the most commonly encountered chemical state. Concentration techniques, therefore, had to be tested with samples spiked with radioactive waste solutions being discharged. Filtration and ion exchange were employed for quantitatively recovering ^{54}Mn, ^{58}Co, ^{60}Co, ^{134}Cs and ^{137}Cs from 400 liters of seawater. Seawater was pumped through

Table 1

Concentration Methods for Radionuclides in Seawater

Radionuclide	Volume Analyzed (liters)	Concentration Method	References
^{32}P	1-10	Extraction into isoamyl alcohol or n-butanol	10,11
Insol. Hydroxides*	10-1000	Coprecipitation with $Fe(OH)_3$ Absorption on MnO_2	11-15
^{58}Co, ^{60}Co	20	Ion exchange on chelating resin	16
^{89}Sr, ^{90}Sr	100-200	Precipitation of Sr as carbonate or oxalate	17-22
^{95}Zr, ^{95}Nb	5	Precipitation as $Zr_3(PO_4)_4$ and $Nb_2O_5 H_2O$	23
^{103}Ru, ^{106}Ru	5-50	Coprecipitation with CoS	24
^{134}Cs, ^{137}Cs	50-200	Absorption on inorganic ion exchangers AMP+ or metal ferrocyanides	25-28
^{239}Pu	1-50	Coprecipitation with $BiPO_4$ or $Fe(OH)_3$	29-31

* ^{51}Cr, ^{54}Mn, ^{55}Fe, ^{58}Co, ^{60}Co, ^{65}Zn, ^{95}Zr, ^{95}Nb, ^{103}Ru, ^{106}Ru, ^{141}Ce, ^{144}Ce

+ AMP: ammonium phophomolybdate

0.45-u membrane filters in cartridges at a flow rate of 15 liters/minute to collect particulate radionuclides. The filtrate was then passed through Chelex-100 chelating ion exchange resin to concentrate ^{54}Mn, ^{58}Co, and ^{60}Co, and through ammonium hexacyanocobalt ferrate (NCFC) on silica gel for ^{134}Cs and ^{137}Cs retention. A disadvantage was the relatively slow (12 liters/hour) flow rate necessitated by the slow exchange rate of the chelating resin.

Selection of the appropriate radiation measurement instrument also contributes to detection sensitivity. For radionuclides that emit gamma rays, the Ge(Li) detector with multichannel analyzer is now usually preferred. The high detector resolution permits simultaneous measurement of numerous photon-emitting radionuclides with precise identification by gamma-ray energy. Moreover, it analyzes the sample nondestructively and can process volumes of several liters. Instruments for radionuclides that emit only alpha particles, beta particles, or weak x-rays have lower limits of detection, but, with few exceptions, chemical separation is needed before measurement.

In situ measurements of beta particles have been obtained with Geiger-Mueller counters, and of gamma rays, with NaI(Ti) detectors, either directly in the water or in accumulation media such as ion-exchange resins. Such systems are more efficient than sampling programs in providing immediate and continuous measurements of radioactivity in water, but the counting efficiency is uncertain, contamination is an ever-present possibility, and the detection sensitivity is usually not as high.

Problems

Difficulties in sampling the marine environment, analyzing samples, and determining radioactivity values are discussed in many of the cited references, particularly reference (3). Acquaintance with this body of knowledge will prevent wasted effort and the dissemination of misinformation. Recommended solutions, however, may only apply to specific sources, samples, and situations. After selecting the monitoring methods that appear to be most suitable, they must be thoroughly tested. The following categorizing of a source-oriented monitoring program is attempted to separate some of these problems for more convenient individual consideration:

1. *Sampling Technique*. If the sampling program is to yield accurate results of average radionuclide concentration or total amount, sampling locations and sampling frequencies

(both horizontal and vertical) must be carefully selected. The sampling program must then be continually evaluated for the influence of differences in sampling locations and times with the best sampling and analysis techniques (see below). As in other monitoring programs, care should be used to insure the integrity of the sample.

2. Sample storage. Radionuclides in a stored water sample may change their chemical or physical form, deposit irreversibly on container surfaces and suspended material, or be lost to the air. Tests may show that the radionuclide or another constituent of the sample should be separated immediately after collection, that a particular container material is suitably inert, or that losses will be prevented by adding acid, base, stable isotopic carrier, or complexing agent. Freezing the sample, lining the container with another material, or analyzing container as well as sample may solve the problem. Although these difficulties may arise with any substance, they are particularly serious for radionuclides at their usual extremely low concentration. Tests must be performed with actual samples, not with radioactive tracers that may be in different chemical forms. It can be anticipated that a process suitable for one radionuclide will not necessarily apply to some others.

3. Radionuclide identification. It is tempting to identify the radioactive constituents of a sample by analyzing a convenient volume with a simple procedure such as gamma-ray spectrometry. However, some radiologically significant radionuclides may be below the detection limit or emit no gamma rays and are overlooked by this procedure. All potentially significant radionuclides should be identified at the source before dilution, when detection is easier, and then measured for confirmation in seawater with appropriate volumes and radiation detectors.

4. Radionuclide concentration and purification. The most effective approach, generally, is to test initially those methods that have been used successfully in similar programs, particularly those evaluated by several laboratories. Even such procedures, however, must be tested with the samples of interest. A different chemical form of the radionuclide in the sample and the presence of interfering substances may invalidate a method that was effective in other situations or had been tested with routinely available radioactive tracers. Hence, the chemical state of the radionuclide must either be determined, or sufficiently altered by the analytical procedure to permit effective treatment for collection, purification, and preparation for measurement. A high yield

in the usual radiochemical procedure of carrier or tracer addition and recovery then provides a useful index of method reliability with regard to radionuclide collection.

5. Radionuclide measurement. Accuracy of results is assured by quality control of the following factors: (a) absence of consistent errors in radiochemical analysis demonstrated by analyzing "known" samples; (b) magnitude of random errors quantified by replicate analyses; (c) level of radioactive contaminants measured by "blank" analyses; (d) calibration of radiation detector for counting efficiency of specific radionuclides or as function of radiation energy with radioactivity standards that are from or traceable to NBS; (e) normal functioning of radiation detection instrument checked by counting performance standards and "background", and (f) identification of radionuclides confirmed by observing decay characteristics such as type and energy of radiation, half life, and genetic relationships. The effectiveness of such programs is enhanced by separating the responsibility for analyses from that for quality control results and participating in intercomparisons with other laboratories, such as the IAEA program (1).

6. Data confirmation. Atypical samples, unknown losses, radioactive contaminants in the environment or the laboratory, and calculational errors are common causes of misinformation in the literature. Most questionable values would be promptly recognized as such by comparison with results for similar samples in the same or other programs, and concentration values in related samples, such as biota or sediment.

7. Data reporting and evaluation. As a minimum requirement, the uncertainty in the results and limits of detection must be reported, and the significance of results discussed in terms of the source, "local background" values, and radiation exposure by critical pathways. Information on other exposure pathways and comparisons with similar situations elsewhere are also useful. Data reports that are undefined with regard to accuracy and radiation impact are at best incomplete and at worst the cause of unending problems.

Summary and Conclusions

An acceptable radiological monitoring program can be prepared on the basis of extensive experience with both routine programs and research concerning radionuclide transport in the marine environment. Many difficulties must be considered: those common to most marine monitoring programs; those associated with the chemical state of the major radionuclides in effluents (including their extremely low concentration); and those related to measuring numerous radionuclides at very low levels. Participation in the program by appropriate specialists is a necessity, and use of only an oceanographer or a radiochemist when both are needed usually leads to initially unrecognized difficulties.

The following activities can be specifically recommended to EPA for assuring an adequate radiological monitoring program:

1. Participation with organizations such as the IAEA to appoint expert groups for designating reference methods for monitoring.
2. A testing program for reference methods to confirm their applicability and observe their limitations.
3. Development of transport models for point sources.
4. Preparation of quality control guides for radiochemical analysis.
5. Support for, and participation in, laboratory intercomparisons of seawater samples with radionuclides of interest.
6. Support for availability of appropriate NBS radioactivity standards in useful forms.

Many of these activities are components of existing programs in EPA, but some might well be expanded.

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METHODS FOR MONITORING RADIOACTIVITY IN AQUATIC BIOTA

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I. INTRODUCTION

The general scope of this paper will be limited to a discussion of monitoring for radioactivity in aquatic biota, and the specific purpose will be: to review pertinent literature concerning monitoring radioactivity in the aquatic environment; to discuss the critical pathway and specific activity approaches to sampling; to outline two monitoring programs conducted by the Laboratory of Radiation Ecology (LRE); and to describe sample collection, sample handling, chemical procedures and radiometric equipment used in monitoring programs now in progress at the Laboratory of Radiation Ecology.

Literature concerning monitoring radioactivity in the marine environment is extensive, and only a brief review will be presented in this report. A principal source of information on this subject is found in the publications of the International Atomic Energy Agency (IAEA). The 1970 IAEA report, "Reference Methods for Marine Radioactivity Studies," details procedures for sample collection, storage and preparation, describes analytical methods for several radionuclides, and is a good general purpose reference manual for monitoring studies. Other general references include "Methods of Surveying and Monitoring Marine Radioactivity," (3); the "Manual on Environmental Monitoring in Normal Operations," (4); "Environmental Contamination by Radioactive Materials," (5); and the Environmental Protection Agency (EPA) technical report, "Environmental Radioactivity Surveillance Guide," (24).

Of the references dealing principally with radiochemical techniques employed in monitoring, the most complete practical survey of procedures available is prepared by the USAEC Health and Safety Laboratory, entitled "HASL Procedures Manual" (23). This document contains information on counting procedures, sample treatment, analytical separations and data reporting. The details of the procedures have been tested by both HASL and contractor laboratories. Other useful documents discussing radiochemical analysis are the 1967 Public Health Service report, "Radioassay Procedures for Environmental Samples", the 1966 World Health Organization report, "Methods of Radiochemical Analysis"; and two IAEA reports, "Quick Methods for Radiochemical Analysis," (6); and "Rapid Methods for Measuring Radioactivity in the Environment," (8).

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Two National Academy of Sciences publications of interest are, "Radionuclides in Food," (14) which discusses sources, levels and does evaluations of both natural and man-produced radionuclides in food and water, and "Radioactivity in the Marine Environment," (13) which provides a summary of what has been learned about the sources, distribution and concentration of radionuclides in the marine environmment and their effects upon marine organisms and man.

II. Monitoring Programs

Sampling and analytical procedures have been developed in our laboratory, or adapted from the procedures of others, to determine the concentrations of radionuclides in many types of environmental samples. (In this regard, the term "environmental samples" refers to all types of samples--plants and animals as well as air, water, soil and sediments.) The procedures have been used for the collection and analyses of a large variety of samples from far-ranging geographical areas. The samples have been obtained in conjunction with the Laboratory's research programs which include monitoring radionuclides at Amchitka Island in the Aleutian Islands, studies of the distribution of radionuclides at the former test sites of Bikini and Eniwetok Atolls in the Marshall Islands, monitoring of the marine environment of Washington State to delineate distribution of radionuclides produced by the Hanford Atomic Works, conducting a survey of a proposed nuclear power plant site, and determining levels of natural lead-210 and polonium-210 in the marine environment.

A. Objectives

A common reason for the initiation of a sampling program is to gather data that ultimately may be necessary to assess the human radiation exposure from radioactive materials released to the environment. Another reason is to make use of the radionuclides that are present in the environment as tags or tracers that can provide basic information about environmental processes, studies that may be, but are not necessarily, related to the assessment of human radiation exposure. In some situations the amount or type of radionuclides released may not be significant enough to justify a monitoring program based on either of the above reasons; however, a monitoring program solely to satisfy public interest as to the fate of the radionuclides released to the environment may still be required. In the opinion of the authors, and others (22, 27), monitoring radioactive waste disposal operations to provide protection for marine resource organisms (salmon, crabs, etc.) is not necessary if ICRP standards for human health are maintained.

B. Approaches

Two of the approaches to a monitoring program concerned with public health are the critical pathway approach and the specific activity approach.

The critical pathway approach is a study of what happens to a radionuclide from the time is is introduced into the aquatic environment until it reaches man, and is described in detail in the International Commission on Radiological Protection (ICRP) Publication No. 7 (9). Briefly, it suggests that if all of the radionuclides present in a particular environment and all of the ways by which they can reach man are investigated, it may be found that only one or two radionuclides and pathways need to be investigated as they will be more critical to human health than all of the other radionuclides and pathways combined.

A schematic outline of the critical pathway approach, as adapted from Preston, 1969, is shown in Figure 1. The figure suggests that there are three steps to be followed in order to determine the maximum permissible amount of radionuclide(s) to be discharged daily as an environmental contaminant. First, the concentration of the radionuclide(s) in the receiving water and in the food web, and the dietary and living habits of the local population are established; second, the critical pathways are identified from the above information; and third, the dose to the human population is estimated, the dose compared to the permissible exposure as defined by the ICRP and the results of the comparison are related to the daily discharge rate.

Normally, in the first stages of a critical pathway monitoring program, subsidiary pathways also will be sampled until enough data are available to confirm or reject selection of the critical pathways. However, by combining present knowledge of the behavior of radionuclides in the aquatic environment with knowledge of local food organisms and local human population habits, a reasonable allocation of sampling effort can be made at an early planning stage.

The concept of the specific activity approach was presented in NAS-NRC publication 985 (12) and has been discussed in several recent publications, including Kaye and Nelson (10), Preston (15), and the NAS RIME Report (13). This approach provides a method for establishing maximum permissible concentration values for radionuclides in seawater (MPCC) and, basically, states that the specific activity of a radionuclide in seawater should be no greater than the specific activity for the same radionuclide in the critical organ of man. Specific activity in this context is defined as microcuries of the radioisotope per gram of element; for example, uCi of ^{55}Fe per g of Fe. For the critical organ of man, the specific activity is the ratio of the maximum permissible burden for the critical organ as defined by ICRP to the weight of the total element in the critical organ; for seawater, the specific activity is the ratio of the MPCC value in uCi per kg to the weight of the total element per kg (of seawater). If the specific activity for seawater and for the critical organ of man are equated, then,

$$\text{MPCC (uCi/kg)} = \text{(specific activity in critical organ)(g of element per kg seawater)}$$

Another presentation of the specific activity approach is the schematic outline prepared by Preston (15) and given in Figure 2. The specific activity in Figure 2 is defined as the limiting specific activity for seawater on the hypothesis that if the specific activity for the critical organ is not exceeded in seawater, then the maximum permissible burden for the radionuclide in the critical organ can not be exceeded in man by eating seafoods, regardless of amount.

ESTIMATED ACTIVITY LEVEL IN WATER AT EQUILIBRIUM FROM A KNOWN DISCHARGE RATE

+

LOCAL DIETARY AND LIVING HABIT INFORMATION

+

CONCENTRATION FACTORS FOR ORGANISMS OR MATERIALS IN CRITICAL PATHWAYS

ACTIVITY LEVELS IN CRITICAL PATHWAYS

+

LOCAL DIETARY AND LIVING HABIT INFORMATION

DAILY INTAKE/DAILY EXPOSURE

+

ICRP MAXIMUM PERMISSIBLE DAILY INTAKE/EXPOSURE

MAXIMUM PERMISSIBLE DAILY DISCHARGE RATE

FIGURE 1. CRITICAL PATHWAY APPROACH TO RADIOACTIVE WASTE DISPOSAL ASSESSMENT (ADAPTED FROM PRESTON, 1969)

Foster, Ophel and Preston (2) made a careful comparison of both the critical pathway and specific activity approaches for determining allowable concentrations of radionuclides in seawater. They pointed out that both approaches require knowledge about the kinds, amounts, and the physical and chemical forms of the radionuclides released, about dilution and distribution of the radionuclides in the receiving water due to mechanical and natural processes, and about the availability of the radionuclides to the biota. However, the critical pathway approach also requires information about the concentration of radionuclides from seawater by marine organisms and about the kinds and amounts of seafoods eaten by the local population, whereas the specific activity approach does not. These are difficult parameters to establish and limit the reliability of the critical pathway approach. However, the specific activity approach provides a very conservative estimate of the allowable concentration of radionuclides in seawater because it assumes that all of man's food comes from the sea and does not fully compensate for the reduction in the specific activity value that occurs as the radionuclide moves from the sea to man. This approach is not fully applicable for radionuclides that do not have a stable counterpart in seawater, the critical organ, or both and is not applicable for radionuclides that have the gastrointestinal tract as the critical organ.

$$\frac{\text{ICRP MAXIMUM PERMISSIBLE ORGAN (BODY) BURDEN OF RADIONUCLIDE}}{\text{ORGAN (BODY) CONTENT OF STABLE NUCLIDE}}$$

MAXIMUM PERMISSIBLE SPECIFIC ACTIVITY IN SEAWATER

•

CONCENTRATION OF STABLE ELEMENT IN SEAWATER

MAXIMUM PERMISSIBLE CONCENTRATION OF RADIONUCLIDE IN SEAWATER

•

EQUILIBRIUM CONCENTRATION FROM UNIT RATE OF DISCHARGE

MAXIMUM PERMISSIBLE RATE OF DISCHARGE

FIGURE 2. SPECIFIC ACTIVITY APPROACH TO AQUEOUS RADIOACTIVE WASTE DISPOSAL ASSESSMENT (FROM PRESTON, 1969)

In practice, both the specific activity and the critical pathway approaches may be used to predict the impact of the release of radionuclides. The specific activity approach may be used to provide a first approximation of the magnitude of the problem but the critical pathway approach is usually selected as the means of precisely defining the limits of the problem. Usually, the program begins with a pre-release general survey of the receiving water and of the local population to establish the existing levels of radioactivity in the environment and to provide information for making predictions about the quantity of radionuclides to be released. Then, after the release of the radionuclides begins, the general survey is repeated and is later followed by a monitoring program that is limited to sampling only the critical pathways that lead to man. Indicator organisms (organisms, preferably sessile, available and abundant, which exhibit high concentration factors and long biological half-lives for the radionuclides of interest) whose radionuclide content may be related to that found in a critical pathway organism may also be sampled. If the predicted pathways and those to the local populations are not confirmed by direct measurements after discharge has begun, an adjustment is made in the release of the radionuclides.

C. Examples

Monitoring programs to delineate the movement of radionuclides in the marine environment have been conducted by personnel of the Laboratory of Radiation Ecology (LRE, previously known as the Applied Fisheries Laboratory and the Laboratory of Radiation Biology) since 1946, when monitoring at the Pacific Test Site, Bikini Atoll, was begun. Some of the programs currently in progress at Bikini and nearby atolls were noted in the introduction. A brief description of two of these programs will be given to illustrate typical monitoring studies.

Eniwetok - In 1946, Bikini and Eniwetok Atolls were selected by the US Government for the testing of nuclear devices. The indigenous Marshallese people were removed from these atolls prior to the commencement of testing, which lasted from 1946 to 1958. Recently, Bikini Atoll was returned to the Trust Territory Government and Eniwetok Atoll will soon be returned. Before the Eniwetok people return to their atolls, a comprehensive radiometric survey of air, water, soil, sediments and biota of the atoll was required to provide data to assess the radiological hazards to people returning to Eniwetok to live. The field portion of this survey was conducted from October, 1972 to February,

1973. It was a joint effort of the Lawrence Livermore Laboratory and a number of other laboratories, including LRE, and was coordinated by the Nevada Operations Office of the US Atomic Energy Commission.

Since the level of environmental radioactivity present at Eniwetok in 1964 and earlier is known from past surveys (26), background information was available to assist in the design of the sampling program. The areas were selected for sampling because they are potential resettlement sites or were previous collection sites. In addition, "control" fish for the aquatic biota program were obtained from Kwajalein Atoll, over 300 miles to the southeast of Eniwetok.

There are more than 700 species of fish at Eniwetok but only a few species of reef, benthic and pelagic fishes were selected for use in this study. The species selected were chosen for one or more of the following reasons: they are commonly eaten by the Marshallese; they are relatively abundant at most collection sites; they are representative of a feeding habit; or there is previous relevant radiometric information about the species. Selected invertebrates were also collected for analysis.

All samples were analyzed for gamma-emitting radionucludes and many for iron-55 (^{55}Fe), strontium-90 (^{90}Sr), and plutonium-239, 240 (^{239,240}Pu).

The results of these analyses and of analyses from the other portions of the survey, along with information on the probable living and feeding habits of the returning Eniwetok people, has provided a basis for calculating dose rates to the people. Dose rates, following varying degrees of clean-up of radiological hazards on the atoll, have been calculated. When both the radiological hazard and the sociological factors are known, then decisions can be made about courses of action to reduce the radiation dose to the returning Eniwetok people.

Washington - The second monitoring program I would like to discuss is a study of the distribution of Hanford-produced radionuclides in marine organisms of Washington. The original plutonium production reactors at Hanford, near Richland, Washington released radionuclides into the Columbia River from 1944 until January 1971, when the last of these reactors was shut down permanently. The distribution of Hanford-produced radionuclides in the marine environment from the mouth of the river--550 km from the Hanford reactors--, along the Washington Coast, in the Strait of Juan de Fuca, and in Puget Sound has been monitored by LRE since 1961. At present, two and a half years after the last reactor shutdown, some radionuclides are still present in water, biota, and sediments, due to recycling of the long half-lived radionuclides. It is expected that within time the levels of most remaining radionuclides will be below the limits of detection.

To determine the distribution of Hanford-produced radionuclides, an extensive preliminary survey was begun in 1961 to establish radionuclide distribution in the marine environment by species, by tissues, by season, and by areas. Samples collected from over twenty intertidal locations along the Washington-Oregon coast between Cape Flattery and Coos Bay, which are 260 km north and 350 km south of the river mouth, respectively. As knowledge was acquired as to radionuclide distribution in the organisms, eight coastal areas were selected as regular collecting stations for invertebrates and algae, and four coastal bays, plus Puget Sound, were selected for oyster collection sites. Plankton and demersal fish were also collected at sites extending 240 km off the coast.

Preliminary results indicated that the distribution of Hanford-produced radionuclides was best monitored by zinc-65 (^{65}Zn) distribution; hence, sampling was focused on those organisms concentrating ^{65}Zn. Although other species were still collected, the organisms sampled at most stations and analyzed routinely were mussels, sea anemones, and selected algae.

This type of sampling program continued from 1961 through 1963, at which time it was felt that the original objectives of the survey had been met. Results are reported by Seymour and Lewis (18).

The program was renewed in 1965, one year after completion of the original program. It was resumed in order to observe changes in the levels of radionuclides in marine organisms associated with the permanent shutdown of the eight Hanford plutonium-production reactors between 1965 and 1971. This program emphasized collection of coastal organisms, especially mussels, which were known to concentrate ^{65}Zn, although other radionuclides were measured.

All samples were analyzed for gamma-emitting radionuclides. Results of these analyses indicated that the amount of ^{65}Zn declined with increasing distance from the river mouth and time after reactor shutdown. Further information on this and other corollary studies can be found in Seymour and Nelson (19), Seymour (17), and in annual reports of the Laboratory of Radiation Ecology to the Division of Biomedical and Environmental Research, US Atomic Energy Commission.

III. Sample Preparation

Field samples are frozen as soon as possible after collection and then stored until prepared for analyses. To prepare for gamma-spectrum analyses, the samples are thawed, dissected if necessary, dried at 95C to a constant weight, ground to a homogeneous mixture in a food blender and then packaged in a standard container for gamma counting. Analyses for alpha or beta-emitting radionuclides requires chemical separation of the radionuclide from an aliquot of the dried, ground sample and deposition of the extracted radionuclide on a planchette for counting. To concentrate a dried sample for radiochemical separation the sample is either dry or wet-ashed. Dry-ashing in a muffle furnace to remove all organic material is easier than wet-ashing but it is necessary to maintain strict temperature control to ensure that the more volatile radionuclides are not lost (11). Wet-ashing is a better technique to get samples into solution, but it is more time-consuming than dry-ashing (21). After ashing, the sample can be dissolved in acids or, if it is still insoluble, a fushion technique with hydrofluoric acid can be used. For refractory radionuclides this is most important, since they often are not soluble in mineral acids.

Once the samples are in solution the various group and specific element separations can be made. Radio-tracers or standard chemical techniques are used to determine the chemical yield. If the concentration of an element is low in the sample, a carrier for the radionuclide must be used. It is important to measure the stable element in each sample in order to determine the amount of carrier needed in the chemical procedure.

Some of the radionuclides not detected by gamma spectrometry but commonly determined by alpha or beta counting at LRE are ^{55}Fe, ^{90}Sr, ^{238,239,240}Pu, and ^{3}H. A brief discussion of the methods used for analysis of these radionuclides will be presented here to indicate those procedures found most useful for aquatic biota.

Because of the low concentrations of ^{90}Sr and Pu expected in marine biota, samples up to 500 g wet weight are concentrated for analysis. To extract ^{90}Sr the HASL "Rapid Technique for ^{90}Sr" is used. This procedure employs solvent extraction of yttrium-90 (^{90}Y) which is measured by liquid scintillation methods. Yttrium carrier is added to measure the yttrium yield on extraction. If the first extraction of yttrium is contaminated by rare earths such as europium-155 or 152, the sample is stored for two weeks and the extraction repeated. Since the europium is removed by the first extraction, the second extraction should yield ^{90}Y alone.

The chemical procedure most suitable for plutonium analysis requires an initial solvent extraction of the trans-uranium elements, followed by selective elution of the Pu, using a method adapted from Buttler (1). With certain samples of high salt content, co-precipitation of Pu with calcium phosphate and ion exchange, using an anion exchange resin, was found to be satisfactory (28). Following extraction, Pu was plated on platinum discs by electrodeposition.

The procedure adopted for ^{55}Fe analyses is the method described in HASL-300 (23). By this method, iron-55 is separated by solvent extraction and is plated on copper discs by electrodepostion.

The LRE monitoring program at Amchitka includes the measurement of ^{3}H in free water. Tritium incorporated in the water molecule is the most mobile of all radionuclides produced in underground nuclear detonations, and hence tritium in water is a monitor of radionuclide leakage from the site of the Amchitka detonations. Because of the difficulty of measuring tritium at low concentrations, the limits of detection commonly reported in the literature are 200 tritium units (1 TU = 3.2 pCi/liter). This limit is not adequuate for a reliable measurement of the present levels of ^{3}H in rainfall, which ranges from 25-350 TU. Lower limits of detection can be attained by a electrolytic enrichment process but this procedure is excessively time-consuming. For these reasons, the Laboratory has adapted the procedure of Sauzay and Schell (16) which provides a relatively simple method for analysis of ^{3}H in free water at limits of detection of about 15 TU. The method, in essence, is simply the distillation of water from the samples of temperature differential and vacuum, mixing of the distillate with Insta-gel, and counting the sample in a liquid scintillation spectrometer. Biotic samples were processed in the same way as the water samples except that the samples were lyophilized rather than heated to prevent decomposition.

The equipment used to measure ^{3}H, ^{55}Fe, ^{90}Sr, and Pu is discussed in the following section.

IV. Counting Equipment

Equipment used in the laboratory to measure radioactivity in aquatic biota samples may be divided into three basic categories, alpha, beta, and gamma or X-ray counters.

Gamma counters commonly used at LRE are sodium iodide (thallium activated) and germanium diode (lithium drifted) detectors, with an intrinsic Ge detector used for selected radionuclides such as americium-241 (^{241}Am) and europium-155 (^{155}Eu). The NaI detectors have a greater efficiency but lower energy resolution for gamma-emitting radionuclides than the Ge(Li) detectors. Hence, the NaI detectors are used for counting samples that contain only a few radionuclides, and especially if the energy of their gamma photons are not similar (e.g., fish muscle containing ^{40}K and ^{137}Cs). Besides having better resolution of gamma energy, a Ge(Li) detector also is capable of efficiently operating over a wider energy range (50 KeV to 2 MeV) than is a NaI detector (250 KeV to 2 MeV). Thus, our Ge(Li) detectors are used for samples containing a mixture of radionuclides which would be difficult to resolve on a NaI detector or for samples containing radioelements (^{241}Am, etc.) emitting gammas of energies below the efficient working range of the NaI detector. One requirement of the Ge(Li) detector system is the need to keep the diode cooled by liquid nitrogen. To obtain the gamma energy spectrum of a sample with a mixture of radionuclides, either a system with a 4"x 5" NaI crystal detector and a 400-channel analyzer or a system with a Ge(Li) diode detector and a Ge(Li) diode detector and 4096-channel analyzer are used. To obtain the gamma count of a sample with a single radionuclide or the total gamma count of a sample with a mixture or radionuclides, a system with a 3" x 3" NaI crystal detector and a single-channel analyzer are used.

The intrinsic Ge detector is used for counting samples that emit low energy gamma photons or X-rays in the range from about 6 KeV to 400 KeV. This system has a low efficiency but excellent resolution and is capable of differentiating between X-ray energies 300 to 700 eV apart, depending on the energy of the X-rays being measured. The diode of this system also must be cooled by liquid nitrogen but only during operation.

A gas proportional tube and a 200-channel analyzer make up a system that is used for iron-55 counting. This system has an anticoincidence shield which results in a low background (2 CPM). The electronic components of this system also are used with an *in situ* underwater NaI probe recently used at Eniwetok to measure gamma-emitting radionuclides in lagoon sediments.

Two types of beta counters are used in our laboratory; one, a total beta system, the other, a liquid scintillation system. The latter is used mainly for counting $^{90}Sr-^{90}Y$ and ^{3}H, for which this system has a low background and high efficiency. The total beta system has an anticoincidence shield that reduces the background to about 1 count per minute and is about 30% efficient for ^{32}P. Beta counts must be corrected for the counts that are absorbed by the sample matrix on the counting planchette. A reliable estimate of this factor, self absorption, may be difficult to obtain.

Two alpha counting systems are used at LRE, a total alpha counter and an alpha diode counter. Using a zinc sulfide screen as detector, the total alpha counting system counts about 50% of all alpha particles emitted by a sample without resolving the energies of the particles. For samples with a single alpha emitting radionuclide the total alpha counting system is used and for samples with more than one alpha emitting radionuclide the alpha spectrometer system is used. By selection of the proper method and equipment, it's possible to measure all types of radioactivity in environmental samples.

V. Conclusions

In conclusion, personnel charged with monitoring a radioactive waste disposal operation from a nuclear reactor or other sources can find relevant information in the published literature as to methods and procedures to be used to quantitatively and qualitatively identify the radionuclides present in the environment. These procedures may have to be altered to fit the particular situation, but reference to the literature and consultation with specialists presently working in the field usually can resolve any difficulties that may arise.

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THE PROPERTIES AND COMPOSITION OF SLUDGES

R. B. Dean and J. E. Smith, Jr. *

Production and Treatment of Sludges

Sludge is a liquid-solid mixture containing contaminants removed from waste water by physical, biological, and chemical treatments. Although sludge contains solids, one problem of its treatment is the removal of water that is in close association with waste solids. The major part of the cost of sludge treatment is directly related to the tons of water associated with each ton of solids. A typical digested sludge contains about 20 tons of water for each ton of solids. A thin, waste-activated sludge from biological treatment may contain well over 100 tons of water per ton of solids (Table 1). Dewatering and drying sludge are expensive operations that can cost as much as $50 per ton of dry solids produced.

The quantities of typical sludges as they are removed from clarifier tanks or thickeners are shown in Table 2. Several types of sludge may be produced at different stages of a conventional waste treatment system. Figure 1 shows a typical flow sheet for an activated sludge plant. A trickling filter plant would use essentially the same flow sheet, substituting a rock or plastic-filled "filter" for the aerator. The biological sludge sloughed off a trickling filter is frequently called humus in England. It is similar but not identical to the organic humus found in good soils.

Raw primary sludge consists of readily settleable organic matter, fine sand, and silt. It is highly putrescible and cannot be stored even for a few hours in warm weather without some type of stabilization to prevent odors from decomposition.

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TABLE 1

WATER CONTENT OF SLUDGES

Treatment	Percent Moisture	Tons of Water/Ton Sludge Solids
Primary Sedimentation	95	19
Chem. Precipitation	93	13.3
Trickling Filters		
Humus - Low Rate	93	13.3
Humus - High Rate	97	32.4
Activated Sludge	98-99	~65.6
Well Digested Sludge		
Primary Treatment	85-90	~7.0
Activated Sludge	90-94	~11.5

TABLE 2
TYPICAL QUANTITIES OF SLUDGE PRODUCED IN WASTEWATER TREATMENT PROCESSES

Treatment	Keefer (1940)	Fair & Imhoff (1955)	Babbitt (1953)	McCabe & Eckenfelder (1963)
Plain Sedimentation	2,950	3,530	2,440	3,000
Trickling Filter Humus	745	530	750	700
Chem. Precipitation	5,120	5,100	5,250	5,100
Activated Sludge	19,400	14,600	18,700	19,400

(Given as gallons/million gallons sewage treated)

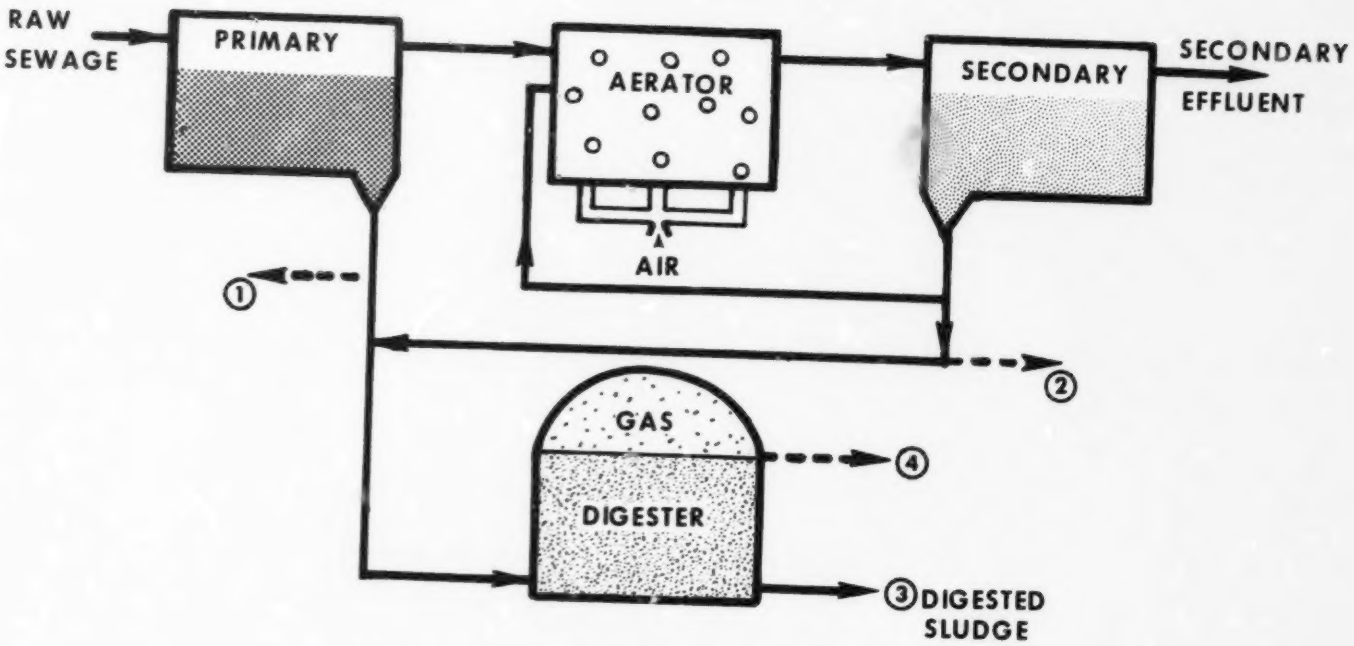
RAW SEWAGE
PRIMARY
AERATOR
AIR
SECONDARY
SECONDARY EFFLUENT
①
②
GAS
DIGESTER
④
③ DIGESTED SLUDGE

Figure 1. **BIOLOGICAL SEWAGE TREATMENT**

Waste-activated sludge (WAS) is the product of biological multiplication of microorganisms feeding on soluble and suspended organic matter in the presence of dissolved oxygen. A major part of the microbial sludge is returned to the aerator; but a fraction, representing net growth, is wasted. Waste-activated sludge is also putrescible. It may be treated separately; it may be combined with primary sludge for further treatment; or it may sometimes be discharged directly to the influent sewer to be collected with the primary sludge.

Most of the bacteria in waste-activated sludge are floc-forming Zooglea, which are related to Pseudomonads. Up to 90 percent of the Zoogleal mass is extracellular jelly secreted as bacterial capsules (4). This gelatinous mass entraps small particles and organisms in flocs that settle with the bacteria, leaving a clarified, final effluent.

Primary sludge, of course, contains high concentrations of fecal coliform bacteria, especially Escherichia coli. It also contains lesser quantities of intestinal and respiratory tract organisms, many of which may be disease-causing (12). Cholera, thyphoid, and dysentary are diseases commonly associated with fecal wastes (7). Amoeboid cysts, intestinal worm eggs, and parasitic fungi may be transmitted by sludge (15). Sewage treatment removes disease organisms from the effluent primarily by sedimentation; however, the organisms may remain in a viable state in the sludge (14).

The high bacterial content of raw sludge causes putrefaction within a few hours because oxygen is used up and anaerobic forms take over. Controlled putrefaction is called anaerobic digestion. If sludge is held for 10 to 30 days in a digester, methane fermentation converts about half of the organic matter to gases. A mixture of methane and carbon dioxide is produced that has about one half the heating value of natural gas and is contaminated with hydrogen sulfide and other odorous substances. There is enough energy in the gas to operate normal mechanical equipment and air compressors in a sewage treatment plant. As digestion converts the solids to gases, the mass of sludge to be treated in subsequent operations is substantially reduced. Well-digested sludge does not putrefy further; it is a dark liquid resembling an emulsion of crude oil and frequently has a similar odor.

Digestion is usually carried out as a semi-continuous process (28). A fraction of the digester contents is withdrawn periodically, usually every day, and the volume is made up with raw sludge. The contents of the digester are mixed by gas evolution, usually supplemented by mechanical mixing or recirculation. If 5 percent of the contents of a digester is replaced each day, the nominal detention time will be 20 days (100%/5% per day); however, 5 percent of the removed sludge will have received only one day's exposure to anaerobic conditions. Digested sludge is frequently thickened by settling and the supernatant foul liquor is returned to the plant for further treatment. Characteristics of digester supernatant, which also represent the composition of the interstitial water in sludge are shown in Table 3.

Because anaerobic digestion is easily upset by a variety of toxic influents, aerobic digestion (or aerobic stabilization) of sludge is sometimes used, especially by small treatment plants because it entails lower capital expenditures. With aerobic digestion in this process, waste sludges are aerated, as in secondary treatment, until a large part of the organic solids have been destroyed (18). There is no generation of fuel gas, and energy is required to provide aeration. The process is inherently more stable than anaerobic digestion, and the final product is also free from further putrefaction.

Although conditions in digesters are unfavorable for the multiplication of most pathogenic organisms, they are not lethal, and the principal bactericidal effect appears to be related to a natural dieoff with time. Kenner et al. (13) have reported on bacterial concentrations in digested primary sludge and undigested waste-activated sludge. Table 4 shows representative data. The large differences between raw waste-activated sludges A and B, which were taken 11 days apart, are not unexpected and probably represent day-to-day fluctuations. Consistent differences between aerobic and anaerobic destruction of pathogens have not been reported.

Phosphates are sometimes removed from sewage effluents by chemical precipitation. Chemicals may be added at any stage of treatment, including treatment of the final effluent in a tertiary process. The added chemicals alter the physical properties of the sludges produced, sometimes favorably and sometimes quite unfavorably. They increase the mass of sludge produced and, of course, also increase its phosphate content. Chemicals that have a significant influence on the bulk properties of sludges include lime, alum, and iron salts. Lime treatment adds calcium carbonate, phosphate, and sometimes magnesium hydroxide to the sludge. Alum and iron salts add the respective aluminum and ferric phosphates and the hydroxides. All three

TABLE 3

PROPERTIES OF DIGESTER SUPERNATANT (MUNICIPAL WASTEWATER SLUDGE)

Item	Standard Rate	High Rate
Total susp. solids (mg/l)	4,000–5,000	10,000–14,000
Total solids (mg/l)	2,000–3,000	4,000–6,000
BOD (mg/l)	2,000–3,500	6,000–9,000
Volatile solids (mg/l)	650–3,000	2,400–3,800
Alkalinity (MO)(mg/l)	1,000–2,400	1,900–2,700
H_2S(mg/l)	70–90	190–440
HN_3-Nitrogen (mg/l)	240–560	560–620
pH	7.0–7.6	6.4–7.2

After Maliva et al.,1971

hydroxides are bulky and hard to filter (1). The same chemicals may also be added to sludge, after it has been separated from the effluent, to condition or stabilize it. Sludge is sometimes treated with large doses of chlorine that produce hydrochloric acid and chlorinated compounds and destroy ammonia and pathogens (9).

Heat treatment of sludge to improve dewaterability is carried out at temperatures above 160° for about half an hour. These conditions will completely destroy all living organisms. If oxygen is supplied together with heat treatment, some of the organic matter may be oxidized; the process is then called wet oxidation. All heat treatment processes increase the concentration of soluble organic matter and ammonia in the supernatant liquor or "soup." This soup, although sterile when it is produced, is a rich nutrient broth that can putrefy if allowed to come into contact with bacteria from the environment. The dewatered sludge is, however, resistant to putrefaction (3, 20).

Lagoon or other storage for many months is frequently depended upon to reduce the numbers of pathogenic organisms, particularly those that cannot multiply outside the human body (10, 14). Storage may be necessary, in any case, if sludge is disposed of only part of the year, and additional storage lagoons can be built into the system to provide more protection against transmission of disease. Because sludge will settle in lagoons to form a mud that may become too thick to pump, resuspension in water or effluent may be necessary before the sludge can be removed from the lagoon.

Composition of Sludges

Sludges analyses are normally reported on a dry weight basis. Only a few substances such as salts and ammonia are soluble in sludge water; when sludge is dewatered, they will be lost in proportion to the extent of dewatering. Most substances in sludge are insoluble or are absorbed on sludge solids; these will be retained when sludge is dewatered unless they are present in the suspended-solids fraction. Many so-called dewatering treatments, especially centrifugation and gravity thickening, produce a centrate or filtrate that contains a significant fraction of suspended solids. The concentrate from a scroll-conveyor, solid-bowl centrifuge may contain as much as 70 percent of the solids in the feed (22). A differently designed centrifuge has achieved 99.9% solids capture with the assistance of polymers (30). Pressure filters can also produce filtrates with much less than 1.0% of the feed solids concentration (8).

Sewage varies in flow and composition from hour to hour and day to day reflecting the living patterns of the population. It also varies from place to place depending primarily on industrial contributions. The normal operation of a sludge digester provides the equivalent of a 10-day moving average of sludge composition, which effectively damps out hourly and diurnal variations. Solids drawn from a sludge thickener or from an inefficiently stirred digester will vary significantly in solids content with time of withdrawal and will reflect variations in solids concentration within the container. Therefore, a carefully designed sampling scheme must be used to obtain a true representation of any given batch of sludge (16). Sludge settles in lagoons leaving a surface layer of oil and a gradient of solids content increasing downward. Since sludge is a non-Newtonian fluid, there will be little or no lateral mixing in lagoons, and significant variations from point to point may be expected in lagoons that have been filled over a long period of time (29). Analyses of multiple samples, rather than replicate analysis of a composite sample, gives confidence in the sampling scheme and provides an estimate of the variability of the data.

Organic matter in sludge consists of food residues, bacterial solids, cellulose, humus-like compounds structurally related to lignin, hydrocarbons, and chlorinated hydrocarbons of low volatility, fragments of plastics and rubber, and miscellaneous synthetics such as adsorbed detergents and paint latex.

Grease in sludge is defined as the material extractable by hexane from an acidified sample under rigorously standardized conditions (26). The grease fraction includes soluble hydrocarbons, fatty acids, waxes, and chlorinated hydrocarbons. The potentially floatable fraction of sludge includes grease and insoluble fragments of rubber and polyethylene.

Inorganic constituents of sludge, with the exception of nitrogen compounds and mercury, may be determined after the organic matter has been oxidized. Dry ashing in a muffle furnace at 450°-600°C or wet ashing in mixtures of oxidizing acids may be used. Major inorganic components of sludge may be similar to those found in soil. Oxides, carbonates, silicates and phosphates of calcium, magnesium, iron, and aluminum make up the bulk of the inorganic fraction of sludge (Table 5).

Sludge contains the major plant nutrients nitrogen, phosphorus, and potassium at levels that are about one-fifth

TABLE 4

BACTERIA IN SEWAGE SLUDGE

(per 100ml)

	Fecal coli (x 10^6)	Salmonella	Pseudomonas
Raw Primary	11.4	460	46,000
Trickling Filter	11.5	93	110,000
Raw WAS - A	2.8	74	1,100
Raw WAS (Thickened) -B	20	9,300	2,000
Raw WAS - C	2.0	2,300	24,000
Anaerobic Digested Primary	0.39	29	34
Aerobic Digested WAS	0.66	150	100,000
Anaerobic Digested WAS	0.32	7.3	1,000
Iron Primary	32	460	21,000
Lime Primary pH 9.0	32	1,500	24,000
Lime Primary pH 11.5	0.014	< 3.0	<3.0

After Kenner, 1972

TABLE 5

SUMMARY OF MAJOR AND MINOR ELEMENTS IN SLUDGE
(mg/g dried sludge)

Element	Primary Sludge	Activated Sludge	Digested Sludge
Aluminum	5.1	10.0	17.9
Antimony	n.a.*	n.a.	0.9
Arsenic	1.2	1.2	n.a.
Barium	2.2	1.2	1.4
Beryllium	0.0025	0.0035	0.0025
Boron	0.10	0.070	0.046
Cadmium	0.19	0.35	0.26
Calcium	n.a.	13.0	33.5
Chromium	2.0	4.3	2.3
Cobalt	0.22	0.002	n.a.
Copper	2.0	1.1	1.6
Gallium	0.06	0.05	0.05
Iron	16.1	40.5	30.6
Lead	1.0	1.5	1.9
Magnesium	10.6	7.0	7.5
Manganese	0.78	0.31	0.98
Mercury	0.005	0.02	n.a.
Molybdenum	0.36	0.20	0.25
Nickel	0.52	0.38	0.38
Phosphorus	3.8	19.9	12.8
Potassium	n.a.	4.2	2.8
Silicon	n.a.	40.0	162
Silver	0.24	0.15	0.20
Sodium	4.0	4.4	6.2
Strontium	o.13	0.16	0.26
Sulfur	n.a.	10.1	12.3
Tin	0.95	0.50	0.60
Titanium	14.8	11.8	14.2
Vanadium	2.1	0.7	5.2
Zinc	6.9	3.3	4.0
Zirconium	1.7	10.0	2.0

*n.a. not available
After Salotto et al., 1971

TABLE 6

MINERAL NUTRIENTS PERCENT OF DRY SLUDGE SOLIDS

Total N	3.5 - 6.4
Organic N	2.0 - 4.5
P	0.8 - 3.9
P_2O_5	1.8 - 8.7
K	0.2 - 0.7
K_2O	0.24 - 0.84

After Peterson, J.R., 1972.

of those found in chemical fertilizers. Table 6 expresses average analyses, as the elements and in agricultural units of sludge. Sludge that has been digested and dewatered by filtration, centrifugation, or by drainage on drying beds will lose to half of its nitrogen as ammonium salts in the removed water. Potassium is also lost when digested sludge is dewatered. Phosphates are present in sludge both as organic compounds and as precipitates with calcium, magnesium, aluminum, and iron depending upon the treatment given. Activated sludge can remove significant quantities of phosphates either as organic phosphate (21) or as calcium phosphate (7). Alum, iron, or lime may be used to precipitate phosphates from wastewater. Digestion will reduce ferric phosphate to ferrous but most of the phosphate remains as the insoluble mineral vivianite (27).

Sludge contains almost all of the metal ions that are discharged to sewers or extracted from plumbing. Heavy metals occuring in sludge in quantities which are significant in foods, include zinc, copper, nickel, cadmium, mercury, and lead. Arsenic, selenium, manganese, and molybdenum may occasionally be present in significant quantities from industrial sources.

The composition of sludges varies so much that meaningful data can only be obtained by applying appropriate statistical methods. The variance or the spread are as important as the average or the geometric mean, and conclusions based on a few spot analyses are likely to be misleading. Obtaining a representative set of samples is perhaps even more important than choosing the best method of analysis. Methods developed for the collection of unconsolidated sediment samples from lakes and marine waters can profitably be applied to the sampling of sludge lagoons.

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MAKING *ARTEMIA* SLUDGE BIOASSAY MORE ECOLOGICALLY RELEVANT

R.J. Nadeau and B.J. Pastalove*

Introduction

The Ocean Dumping Final Regulations and Criteria, under the Marine Protection Research and Sanctuaries Act of 1972, requires chemical and physical analysis of the waste materials as well as a bioassay using an appropriate sensitive marine species (Federal Register, Oct. 15, 1973). Obviously, the most appropriate species would be those endemic to the area of immediate impact of open ocean waste disposal, namely the pelagic or open water plankton.

The microcrustacean *Artemia salina* was selected as the organism of choice for the bioassay requirements because, unlike most endemic zooplankton, it can be reared and maintained under laboratory conditions. *Artemia* is readily available at most pet stores, easily cultured at room temperatures, and has been used as a bioassay organism in the past. *Artemia* does not occur naturally within the marine environment. Within the United States, it exists as disjunct allopatric populations in Great Salt Lake, Utah, and San Francisco Bay, California. In tropical areas it is often found in hypersaline impoundments (salinas) that are used for generating commercial grade salt.

Essentially, the utility of the *Artemia* bioassay lies in its capacity to rank the toxicity of materials destined for ocean disposal. The problem with the *Artemia* bioassay is that it is being used as a partial measure of the environmental impact of disposed materials.

The Interim Criteria limits the permissible concentration of wastes being disposed of in the mixing zone to 0.01 of the TL of the appropriate sensitive marine organism (i.e., *Artemia*). The application factor (0.01) is derived from recommendations of the National Technical Advisory Committee (1).

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No bioassay information is available on municipal sewage sludge using endemic pelagic zooplankton species, except for the preliminary work performed by the Sandy Hook Marine Laboratory for the Army Corps of Engineers (2).

A series of experiments were conducted to determine the relative sensitivities of Artemia and the microcrustacean zooplankton species endemic to the municipal sewage sludge dump site within the New York Bight.

Materials and Methods

Standard Bioassay Method

The bioassay used in this study was an adaptation of the standard method developed by Tarzwell (3) for determining oil and oil dispersant toxicity levels of Artemia salina.

The standard method can be summarized as follows:

1. A. salina eggs from the San Francisco Bay area are hatched in a tray and collected by drawing them to a light; 20 Artemia are transferred by means of a pipette to holding dishes, along with 20 ml of artificial seawater (pH 8.0 - 8.2; salinity 30°/oo) and held for 24 hours.

2. Five concentrations of toxicant, each with five replicates and a control, plus five concentrations of a standard toxicant, sodium dodecyl sulfate USP grade (DDS) are prepared, for a total of 35 dishes.

3. Working from a stock solution, the proper amount of toxicant needed to produce the answered concentrations in a total test volume of 100 ml is drawn off and added to Carolina culture dishes (3.5 x 1.5 in.) along with 80 ml of artificial seawater; with the addition of 20 Artemia and 20 ml seawater, the total volume equals 100 ml.

4. The dishes are incubated at 20°C for 48 hours, at which time the numbers of live and dead individuals are determined for each culture dish and TL values are calculated by interpolation according to Standard Methods (4).

Standard Versus Modified Bioassay Method

In several instances, either modifications or additions were made to the standard method proposed by Tarzwell (3). They include:

1. The use of natural seawater instead of standard artificial seawater to avoid subjecting the delicate indigenous species to the stresses of salinity and pH changes; and using the natural seawater on Artemia to eliminate salinity and pH as variables.

2. The use of an aeration hatching system for Artemia.

3. The use of Artemia larvae (see Figure 1) 5 to 10 hours after hatching versus Tarzwell's use of 24 hour larvae.

4. The use of 10 individuals in each test dish rather than 20 to ease the counting, which became tedious because the Artemia became buried in the highly concentrated sludge.

5. The monitoring of pH, temperature, salinity and dissolved oxygen.

6. The use of a shaker table aeration system to compensate for the high oxygen demand of sludge (an air hose pump system would have been to unwieldly as well as too time consuming to install).

7. The modification of counting procedures to accommodate the special problems of working with heavy sludge concentrations, e.g., allowing the sludge to settle, using the phototactic response of Artemia, searching with the light beam, etc.

Modified Bioassay Method

Experimental Organisms:

Only San Francisco Bay area Artemia salina eggs were used. They were hatched in a 1-liter funnel with a continuous attached air supply; the eggs were kept in constant motion during this period.

Indigenous zooplankton were collected at two sites. The first was an offshore area approximately 4 to 6 miles east of the Shrewsbury Rocks, Sandy Hook, contiguous to the sludge dumping grounds. The second site was a land station in the Shrewsbury River, a tidal river on the Jersey shore. Thirty gallons of water were collected during each sampling period and these periods occurred five times over a 10 week span. Salinity and temperature readings were also made during collection.

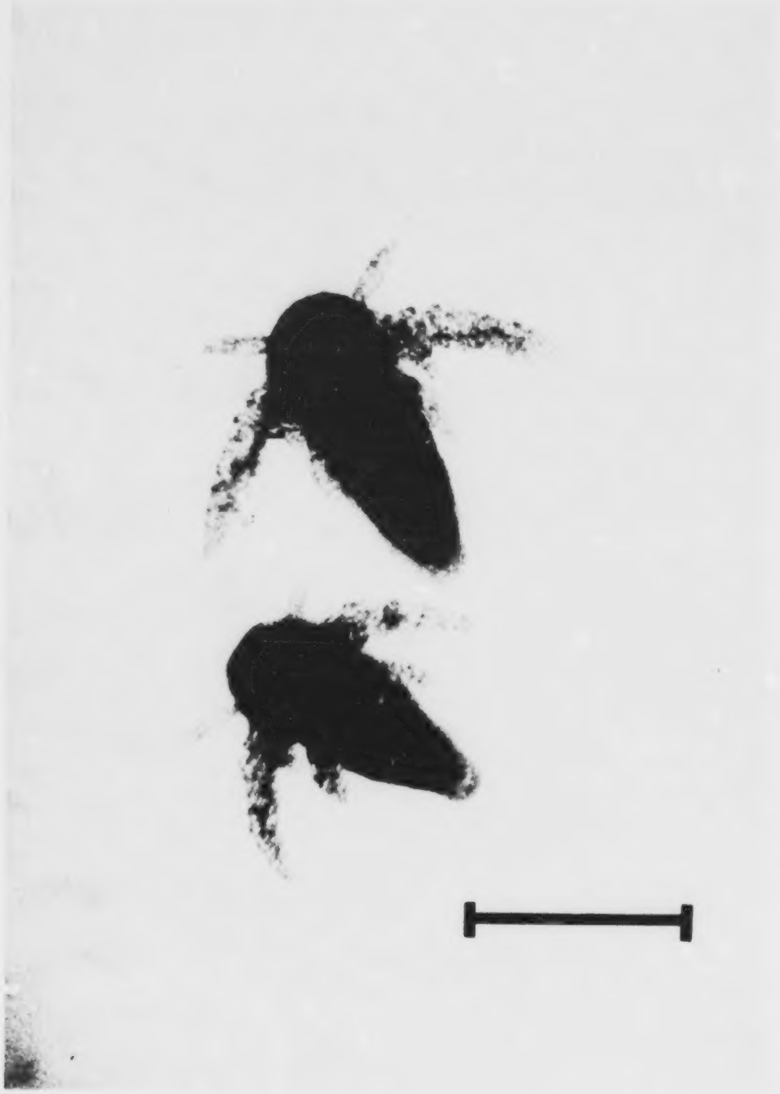

Figure I.

Artemia salina nauplii larvae (5 - 10 hours) 3.1 cm = 50μ) 630x.

The organisms were transported to the laboratory in cooled aerated containers to ensure their viability (temperatures during transport were 20 ± 2°C) and were kept in the laboratory in 20 gallon tanks (18 ± 2°C) with a constant air supply. Other than for the phytoplankton already in the tanks, the organisms were not fed.

Age of Organisms:

For Artemia, a period of 24 to 30 hours elapsed between the time the eggs were first placed in the hatching apparatus and the beginning of the bioassay.

With the endemic species, age determination was not possible, although efforts were made to select mature adults only. Generally, organisms were used 2 days following their collection, but if a sufficient number of individuals survived until the following week (as usually happened), then a second run was made on the same species.

General Procedures:

For both Artemia and the endemic species, either four or five concentrations of the standard toxicant, DDS were prepared for a maximum total of 72 dishes.

The Carolina culture dishes used as test containers were washed in detergent, rinsed with hot water, and oven dried.

Preparation of Test Concentrations:

From the results of preliminary screening tests, a general range of toxicant (sludge) concentrations to be used on Artemia and on the endemic species was chosen. For Artemia, the range was 10^3 to 10^5 ppm (see Figure II) and for the natural species, 1 to 10^3 ppm. A sample of municipal sewage sludge from the Middlesex Regional Sewage Plant, South Amboy, New Jersey, was kept under refrigeration (2°C) in a plastic (Nalgene) container and used during the 2-month testing period. This was done to avoid the inherent variability of using different samples of sludge (5). Physical and chemical analysis of Middlesex sludge is included in Table 1.

Approximately 8 liters of seawater (collected during the sampling periods) were filtered through 0.4u membrane filters (Millipore) to remove phytoplankton and detritus. This water was then used in preparing the test concentrations.

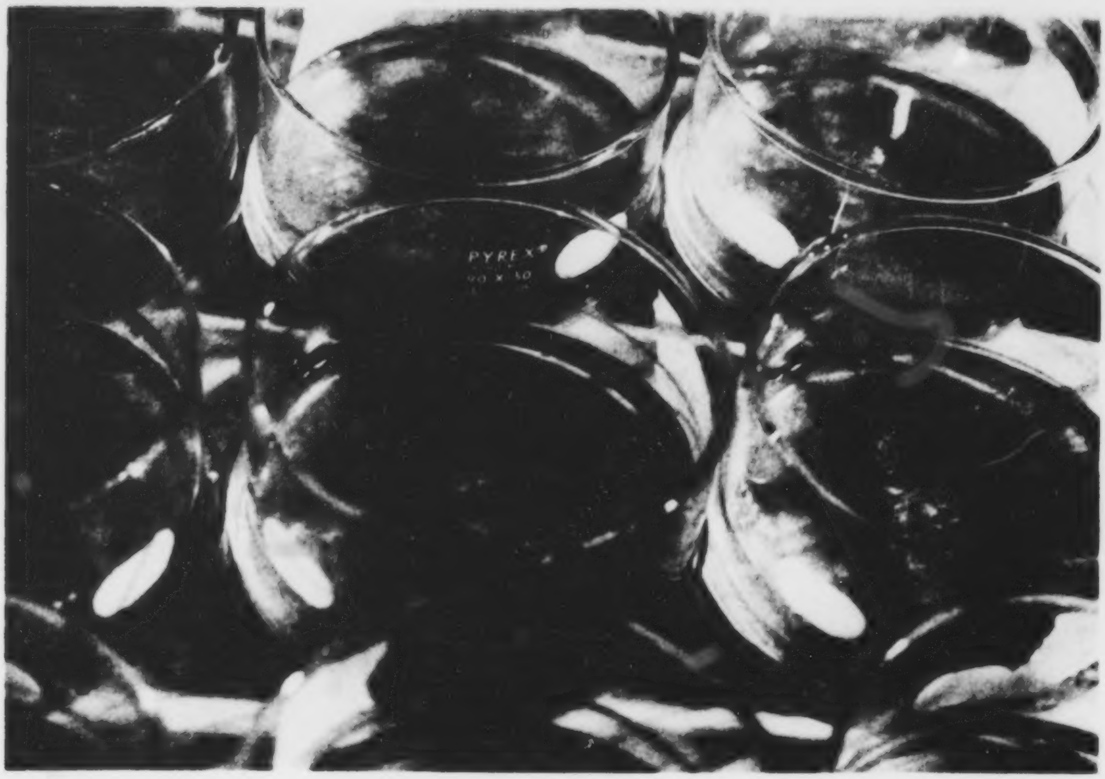

Figure II.

Heavy concentrations of sewage sludge in Carolina culture dishes during bioassay.

PHYSICAL AND CHEMICAL ANALYSIS OF SEWAGE SLUDGE FROM MIDDLESEX COUNTY SEWAGE TREATMENT PLANT TO BE BARGED TO THE DUMPING GROUNDS

Parameter	Sampling Data	
	1 June '73	6 June '73
pH	5.6	5.1
COD mg/l	69,000	87,000
acidity (supernatant) mg/l (CaCO)	1,400	2,110
" (diluted) mg/l (CaCO)	1,350	1.260
alkalinity (supernatant) mg/l (CaCO)	3,770	3,200
" (diluted) mg/l (CaCO)	6,400	5,870
total solids mg/l	50,000	67,000
total volatile solids %	67.3	69.0
suspended solids mg/l	-	54,300
suspended volatile solids %	-	77.6
phenols mg/l	-	15.6
total phosphate-P mg/l	470	633
total sulfide mg/l	16.0	27.2
total Kjeldahl-N mg/l	-	1,400
ammonia-N mg/l	-	169.0

Heavy Metal Analysis from a Composite of the Above Samples Plus Two Earlier Samples

Parameter (heavy metal)	Concentration (mg/l)
Arsenic	0.18
Beryllium	<0.02
Cadmium	6.1
Chromium	3.8
Copper	16.5
Lead	11.8
Mercury	2.6
Nickel	2.42
Selenium	0.015
Zinc	38.5
Vanadium	<0.2

A stock solution of 10^5 ppm concentration of sludge was volumetrically prepared: 100 ml of sludge were removed from the container with a wide-bore pipette and added to 900 ml of seawater. From this stock solution, various dilutions were made; and 100 ml of the appropriate concentration were dispensed in properly labeled test dishes.

Standard toxicant solutions were prepared in a similar manner. The ranges used were 1 to 10 ppm for *Artemia* and 0.1 to 3.0 ppm for the endemic species.

Introduction of Test Organisms:

The previously prepared *Artemia* were counted out with a small pipette and 10 individuals were transferred to each sample.

A sample of the zooplankton was removed from the holding tank and individuals of the most prevalent species were pipetted into each test dish.

Aeration:

To ensure that oxygen levels in the test dishes remained greater than 4 ppm, a shaker table apparatus (see Figure III) was designed, on which all the culture dishes could be placed. The combination of a relatively large surface area and a constant mixing action from oscillation was found sufficient to maintain dissolved oxygen concentrations above 4 ppm.

Exposure:

The entire test ran for 48 hours at a constant temperature of 20±1°C (temperature controlled room) under a regime of 8 hours of low light and 15 hours of dark. The shaker table aeration system was operating at all times.

Observations:

Four physical parameters (dissolved oxygen, salinity, temperature and pH) were monitored at the start of each test run, after 24 hours, and again after 48 hours at the conclusion of the test. A dissolved oxygen probe (Yellow Springs Instrument Co., Model 54), an index refractometer (American Optical Co.), a glass thermometer, and a pH meter (Beckman, Model 96) were used to monitor the four parameters respectively.

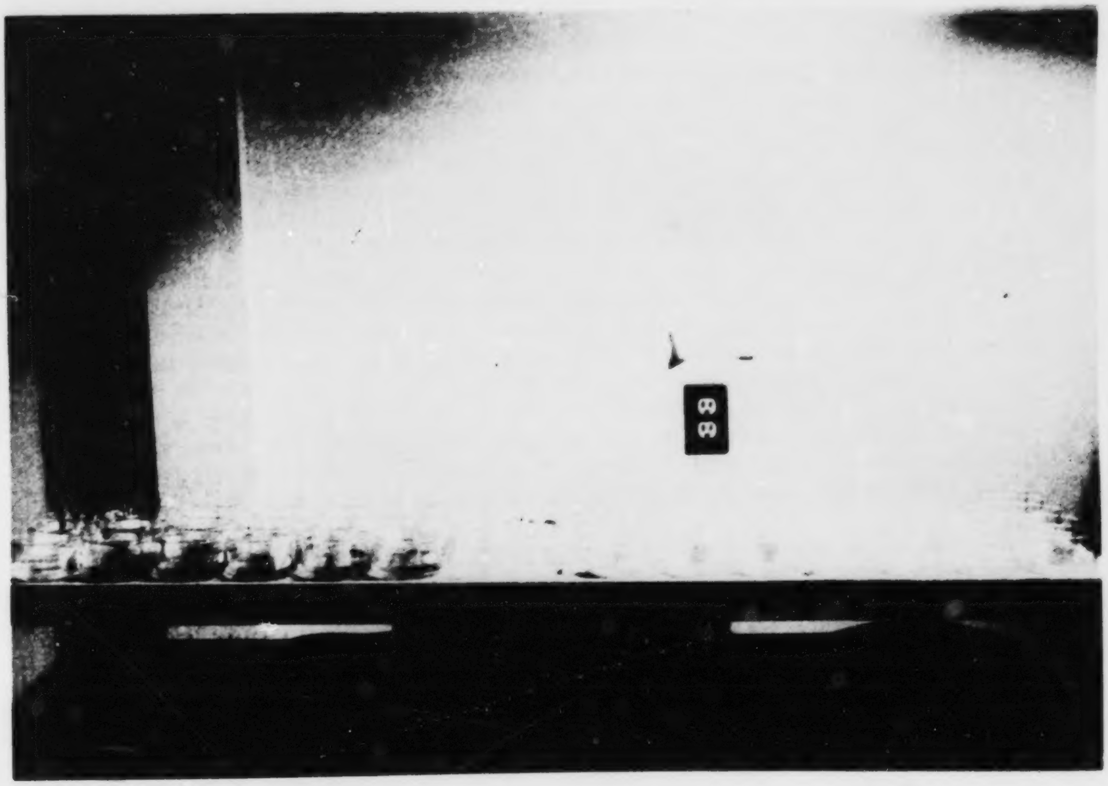

Figure III.

Shaker table assembly for holding carolina culture dishes during bioassay.

TL_{50} values at both 24 hours and 48 hours were determined by counting only the living organisms in each test dish.

Counting Artemia samples (see Figure IV) was especially difficult because of the high concentrations of sludge being used. The general procedure consisted of a 5-minute settling period after which the samples were placed on a black surface (for greater contrast) and a strong beam of light was held at the side of the dish. Individuals in the supernatant fluid swam towards the light source where they could be counted. The procedure became tedious because, at higher sludge concentrations, most of the Artemia were buried within the settled solids. To reach the light source they had to first work their way out into the supernatant fluid. This process, in some cases, took upwards of 5 minutes. By illuminating the side of the dish for a long period of time and then carefully searching the top of the settled material, the sides of the dish, and the mid-depths with the light beam, most individuals could be counted.

The TL_{50} values obtained after 24 hours were not as accurate as those after 48 hours because it was difficult to count moving nauplii. At 48 hours, when the run was terminated, the nauplii were removed as they were counted, and the accuracy of the count was thus improved.

The nature of the sludge made it impossible to find dead individuals, much less probe them to detect movement. Therefore, for the purposes of this test, a positive phototactic response was the criterion for survival, i.e., those individuals that were unable to emerge from the settled material in response to the light source were considered dead.

The method used to count Artemia was also used for the endemic species. There was comparatively little solid matter in suspension (that range of sludge concentrations was lower than that for Artemia), and it was easier to locate live individuals in the reflection of the light beam. However, unlike Artemia, which were strongly attracted by the light source, the endemic zooplankton exhibited only a weak phototactic response. This necessitated a thorough search of all areas of the dish (especially the edges) while trying to keep track of the rapidly moving individuals.

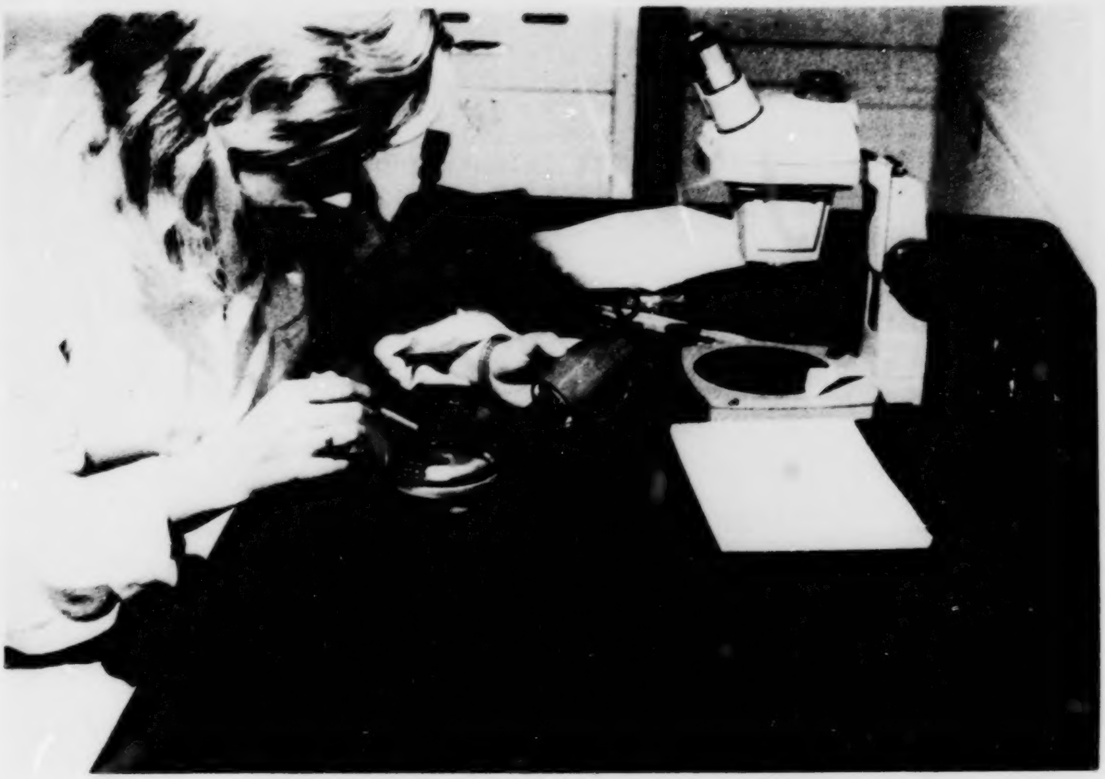

Figure IV.

Counting Artemia nauplii using a pencil light source.

Calculations:

TL_{50} values were calculated by plotting on semi-log paper, the concentrations at which greater than 50 percent and less than 50 percent survived (y axis) and the percent survival at these two concentrations (x axis). A straight line is drawn between the two points; where the straight line crosses the 50 percent survival line is the TL_{50}. Where possible, TL_{50}'s for 24 and 48 hours were calculated for Artemia and endemic species.

Timetable:

An entire 48-hour test run, with preparation, took a total of 5 days. A general timetable is as follows:

Day 1: Field collection of zooplankton sample and seawater.

Day 2: Prepare new batch of Artemia

Filter collected seawater

Decide on range of sludge concentrations to be used

Label clean glassware

Prepare concentrations and set up test apparatus

Day 3: Place Artemia in test dishes

Collect endemic zooplankton from holding tank and place in test dishes (1 to 4 hours depending on species abundance)

Take readings of physical parameters of representative concentrations (i.e., control, highest concentration, and median concentrations)

Day 4: Make 24-hour survival counts (2 to 4 hours)

Take readings of physical parameters

Day 5: Make 48-hour survival counts (2 to 4 hours)

Take readings of physical parameters

Addendum to Method

For the last two bioassays (September 1973), at high sludge concentrations an attempt was made to use the supernatant liquid as the testing media. The method involved preparing an excess of 200 to 400 ml of the solution (i.e., at 7×10^4 ppm where 500 ml of liquid was needed, 800 ml was prepared), allowing the solid material to settle out for 1/2 hour, and then carefully removing the supernatant liquid. The resultant medium is much clearer than the original (though still fairly murky), with much less solid material in suspension, and, therefore, the counting procedure is facilitated.

Results

Toxicity

The early spring tests using the modified *Artemia* bioassay procedure with *Artemia* and a naturally occurring coastal water calanoid species, *Centroroges hamatus*, provided an initial indication that the endemic forms were more susceptible to the toxic element of the sewage sludge (Table 2) than was *Artemia*. Further tests on other indigenous microcrustacenas confirmed this--all endemic species used in this study exhibited a greater sensitivity to sludge toxicity than did *Artemia* (Table 2).

Interestingly enough, except for the last run, the 48-hour TL_{50} for *Artemia* increased with each run during the summer. Revisions of the sludge concentration range were necessary to accommodate this change. These revisions made it increasingly difficult to monitor the target response of the organisms within the higher concentration. Quiescent settling of the solids was required to discern any movement of endemic microcrustaceans and also to allow the *Artemia* nauplii to crawl out from the solids and move towards the pencil light.

Replicate runs with *Paracalanus parvus* showed a similar trend of decreased sensitivity. The August runs, using the same sludge and same population, produced lower TL_{50} values than did those in September.

The reasons for the decreased sensitivity of *Artemia* and *Paracalanus* are unknown but probably are related to subtle changes in the chemical composition of the sludge even though it was stored at 2°C in a dark container for the duration of the studies.

TABLE 2

MEDIAN TOLERANCE LIMIT (in ppm) FOR 50 PERCENT OF TEST POPULATIONS AFTER 24 HOUR AND 48 HOUR EXPOSURE TO SEWAGE SLUDGE

Date	Temperature °C	*Artemia* nauplii 24 hour	*Artemia* nauplii 48 hour	Indigenous Species 24 hour	Indigenous Species 48 hour	Indigenous Species Name
April '73	3.0	all alive	all alive	200	all dead	*Centropages hamatus*
April '73	23.5	all alive	12,000	100	all dead	*Centropages hamatus*
July '73	20 ± 1.5°C	all alive	22,500	15.0	4.4	*Acartia tonsa*
Aug. '73	20 ± 1.5°C	all alive	31,000	49.0	32.5	*Paracalanus parvus*
Aug. '73	20 ± 1.5°C		*	16.0	2.0	*Paracalanus parvus*
Aug. '73	20 ± 1.5°C		**	All alive	330.0	Unidentified harpacticoids
Sept. '73	20 ± 1.5°C	all alive	15,075	Less than 500.0	500.0	*Oithona similis*
				370.0	205.0	*Paracalanus parvus*

* 50% mortality did not occur with 48 hours; 46% mortality in 5 x 10^4 ppm

** 50% mortality did not occur within 48 hours; 34% mortality in 7 x 10^4 ppm

TABLE 2

MEDIAN TOLERANCE LIMIT (in ppm) FOR 50 PERCENT OF TEST POPULATIONS AFTER 24 HOUR AND 48 HOUR EXPOSURE TO SEWAGE SLUDGE

Date	Temperature °C	*Artemia* nauplii 24 hour	*Artemia* nauplii 48 hour	Indigenous Species 24 hour	Indigenous Species 48 hour	Indigenous Species Name
April ' 73	3.0	all alive	all alive	200	all dead	Centropages *hamatus*
April '73	23.5	all alive	12,000	100	all dead	*Centropages*

Table 3 summarizes the sensitivity of each species compared with that of *Artemia* as determined from the experimental runs completed through 48 hours. Those species that had replicate runs showed a wide range of sensitivities. In part, this could be attributed to the increasing TL_{50} of *Artemia* (Table 2).

It is difficult to hypothesize which substances (Table 1) in the sludge are actually responsible for toxicity without a complex factoral experimental design using pure constituents in various concentration combinations. This was neither practical nor necessary for the preliminary study presented here but should be investigated, particularly to stimulate advancement of sewage treatment technology.

Modifying Factors

Temperature:

The temperature-controlled room in which the bioassays were run, kept thermal changes at a minimum; water temperatures during all bioassays were 20 ± 1.5°C (the recommended temperature regime is 20 ± 1°C).

During any one bioassay, there were small increases in temperature (less than 2 degrees, C) over the 48-hour period. However, observed fluctuations in temperature such as these would not significantly affect the TL_{50} values (6).

Salinity:

As a result of evaporation, the salinity increased from the initial to the 48-hour reading (generally salinity was 3 to 7 °/oo greater at the end of the bioassay). Although this is actually a fairly steep increase, the results should not have been significantly affected because:

1. The evaporation was slow enough to let all the organisms adapt to the change.

2. *Artemia* is certainly resistant to high salinity.

3. Estuarine microcrustaceans can tolerate high salinity even though they are not usually found in such waters. Their absence does not result from an inability to withstand higher salinities, but from their inability to sucessfully compete with larger oceanic forms.

TABLE 3

SENSITIVITY FACTORS RANGE FOR INDIGENOUS SPECIES
(TL *Artemia* 48 hour/TL Indigenous Species 48 hour)

Name	Sensitivity Factors
Paracalanus parvus (Claus)	73 - 953 (100 - 1000)
Acartia tonsa Dana	35 - 5113 (50 - 5000)
Unidentified Harpacticoid	212
Oithona similis Claus	30
Centropages hamatus (Lilljeborg)	120
Penilia avirostris Dana	Ultra sensitive (could not be maintained in laboratory)

4. Those species indigenous to open coastal waters can tolerate high salinity levels.

Dissolved Oxygen:

The dissolved oxygen levels in all but the very highest sludge concentrations (>50,000 ppm) were greater than 4 ppm (the recommended "critical" level).

Within the controls, DO levels were constant and high (> 6 ppm). Within the toxicant concentrations, DO levels were lower (which could be related to the high oxygen demand of sludge) but increased during the bioassay. Critically low initial dissolved oxygen readings occurred at 5×10^4 ppm (DO < 3 ppm), 7×10^4 (DO < 2 ppm) and 10^5 ppm (DO < 2 ppm), but by the end of the 48-hour period, levels had increased to greater than 4 ppm.

Discussion

From our study it is obvious that the endemic microcrustacean species, when exposed to the sludge concurrently with the nauplii, are more sensitive to the Middlesex County municipal sewage sludge than are *Artemia* nauplii.

Researchers at the Sandy Hook Marine Laboratory (2) exposed copepods (species not delineated) to sewage sludge at concentrations equal to or greater than those expected under present normal ocean dumping procedures (results are summarized below).

Concentration (ppm)	Results Duration time = 72 hours
10,000	95% mortality after 2 hr.
5,000 3,334	50% mortality after 2 hr.
1,000	No mortality

No mortality was observed at 1,000 ppm (2 to 10 times the TL_{50} concentration derived from our studies). As stated before (see Materials and Methods), municipal sewage sludge varies greatly in composition, particularly from plant to plant. The sludge in the Sandy Hook studies came from the municipal sewage treatment plant at Red Bank, New Jersey. It did not contain any of the industrial waste components treated by the Middlesex County Treatment Plant. The difference in sludge composition could be responsible for the difference in results.

The copepods and cladocerans, being ecotypes to *Artemia* occupy important ecological niches as filter-feeding primary consumers within the pelagic community and as food for all the zooplankton-grazing fish species.

Centropages hamatus (Lilljeborg), the calanoid copepod species used initially to discern a sensitivity difference between *Artemia* larvae and endemic microcrustaceans, is a spring-summer form with wide salinity tolerance (7). It was the largest copepod used in the study and comprises an important bulk of the filter-feeder biomass during its peak abundance. Distribution and abundance studies indicated *Centropages*, including a congeneric form *C. typicus* (see Figure V)., to be the fourth most abundant copepod genus in the New York Bight (9 to 14 x 10^4 individuals per cubic meter) (2).

Paracalanus parvus (Claus) (see Figure VI), the smallest calanoid used in the studies, ranks second in abundance; it occurs throughout the year, and is most abundant in the New York Bight during the fall (20 x 10^3 individuals per cubic meter) (2). The animals used for this study were collected from waters adjacent to the sewage dumping site. Because *Paracalanus* survived well under laboratory conditions, replicate runs on this species, using the initial collection, were possible when additional offshore collecting was not feasible. However, *Paracalanus* showed a wide range of sensitivity to the sludge, and was most sensitive in the early tests. It is possible that the organisms used in the last test were more tolerant (TL_{50} = 10 times that of the most sensitive test), the more sensitive individuals having died during the 2 weeks the population was held in the laboratory.

Acartia tonsa Dana (see Figure VII), a temperate-tropical calanoid form dominant during the summer in Middle Atlantic coastal waters, was collected from the Shrewsbury River, a tidal river on the New Jersey shore. This inshore collection site provided an abundance of individuals when offshore collecting trips were not possible. *Acartia* is tolerant to estuarine-marine conditions and is abundant during the summer and fall (7). This species is relatively sensitive to municipal sewage sludge (TL_{50} - 4.4 at 48 hours) especially when compared with *Artemia*.

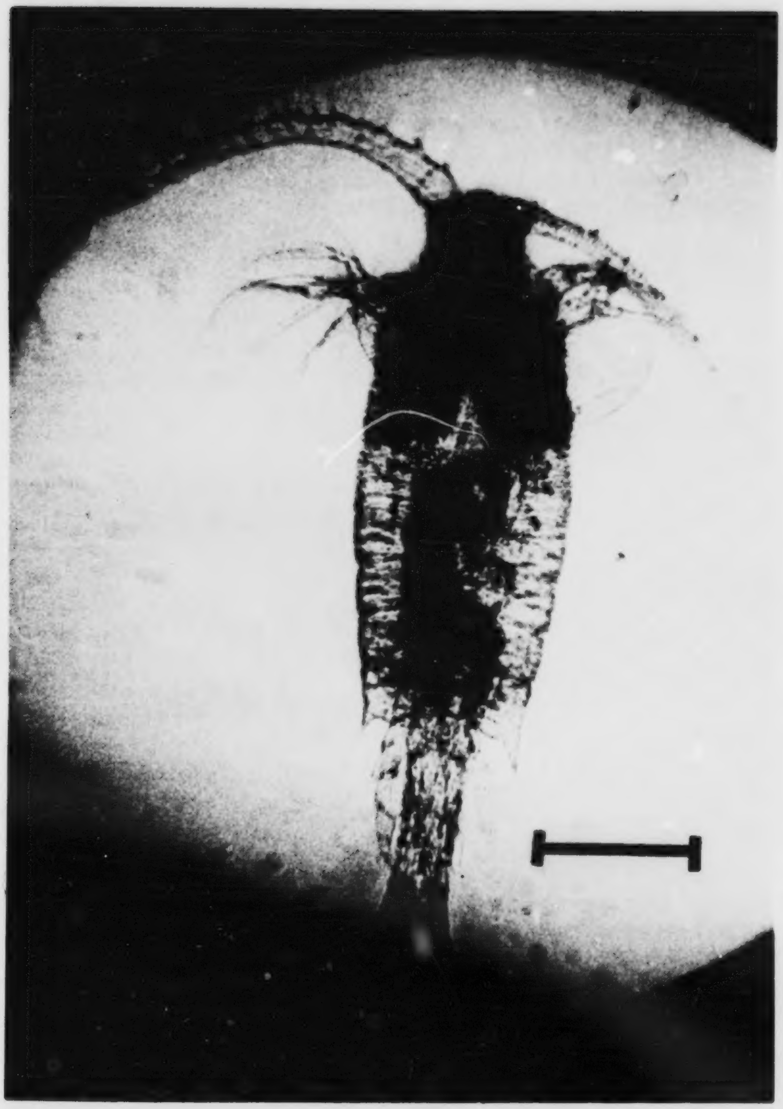

Figure V.

Centropages typicus Kroyer, a calanoid copepod congeneric to C. hamatus, seasonally abundant in the New York Bight (2.2 cm = 50μ) 435x.

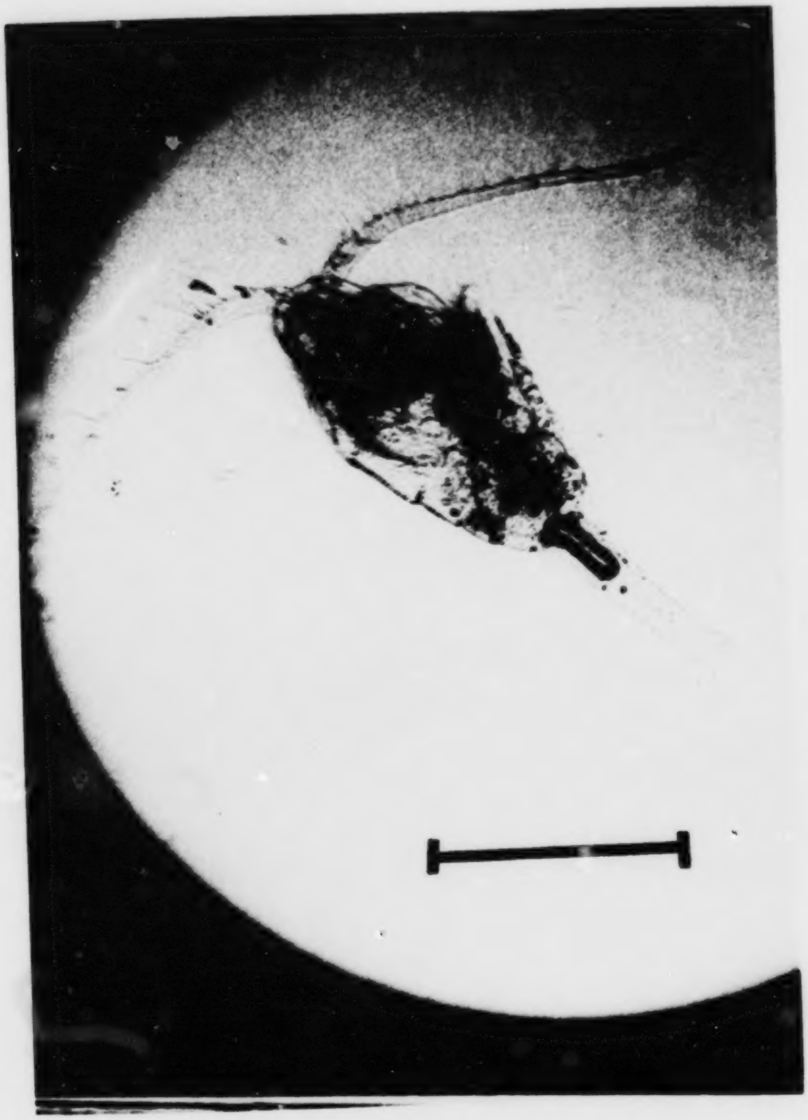

Figure VI.

Paracalanus parvus (Claus), a small calanoid copepod, seasonally abundant in the New York Bight (3.6 cm 50μ) 720x.

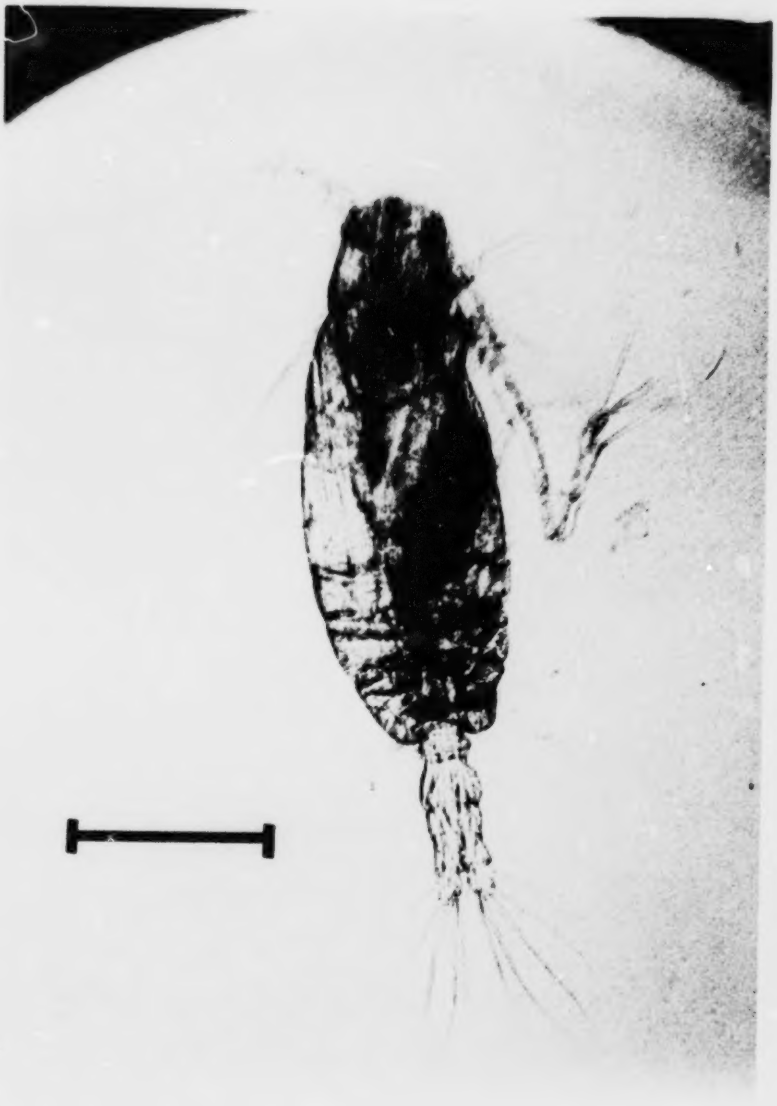

Figure VII.

Acartia tonsa Dana, a calanoid copepod, abundant during the summer and fall in New York Bight (2.8 cm = 50μ) 560x.

Oithona similis (Claus) (see Figure VIII), the only cyclopoid copepod used in the experiment, was collected offshore in late August. This genus is the most abundant and frequently occurring copepod in the New York Bight. It occurs year round and has peak abundance during July (2). Wilson (8) reports that O. similis is littoral and is often found in tidal pools and salt ponds. Oithona ranks high in abundance, but probably is an unlikely food source for zooplankton-eating fish due to its small size (2).

Although not as sensitive as some of the other endemic species, Oithona must still be considered a very critical species due to its great abundance and frequency of occurrence. An impact upon this species could detrimentally influence the pelagic ecosystem of the New York Bight.

An unidentified harpacticoid species (see Figure IX) was used for one of the bioassay runs. Although not as sensitive as some of the other endemic species, it was still more sensitive to sludge than Artemia. Harpacticoids are commonly associated with the mud-water interface of the benthos; but they are also adventitious plankton dwellers, particularly in the detritus-laden waters of the Middle Atlantic Coast (9). These forms can become abundant in the coastal bays and inlets (10). Unfortunately, little is known about harpacticoids as a group, particularly in the taxonomic areas, and workable indentification keys are not available.

A marine cladoceran, Penilia avirostris Dana (see Figure X), was used during one of the tests. Heavy mortalities (80%-90% after 24 hr.) occurred within the control group and invalidated the bioassay. Penilia is also a filter feeder, but it is more sporadic in occurrence and abundance in the Middle Atlantic coastal waters than are the endemic copepod species. Marine cladocerans, like harpacticoid copepods, are adventitious plankton forms but are sporadically abundant (7). In the New York Bight, Penilia and Podon occasionally dominate the zooplankton during July and August (2).

Experimental conditions of the bioassay certainly do not replicate the environmental and ecological variables present in the natural planktonic ecosystem of the New York Bight, and it would be presumptuous to extrapolate from these lethal concentrations for each species to the real world situation. At best, these tests provide the information needed to rank the sensitivity of indigenous species to Middlesex County sewage sludge.

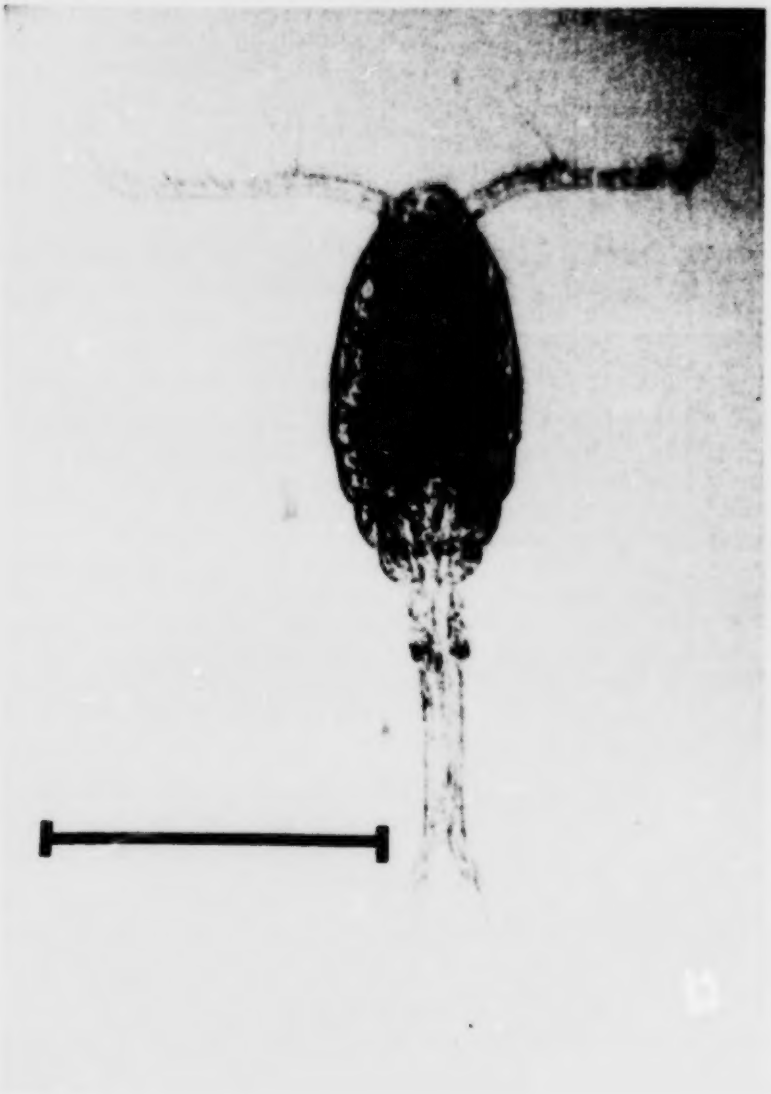

Figure VIII.

Oithona similis Claus, a cyclopoid copepod, the most abundant year round microcrustacean in the New York Bight (4.9 cm = 50μ) 990x.

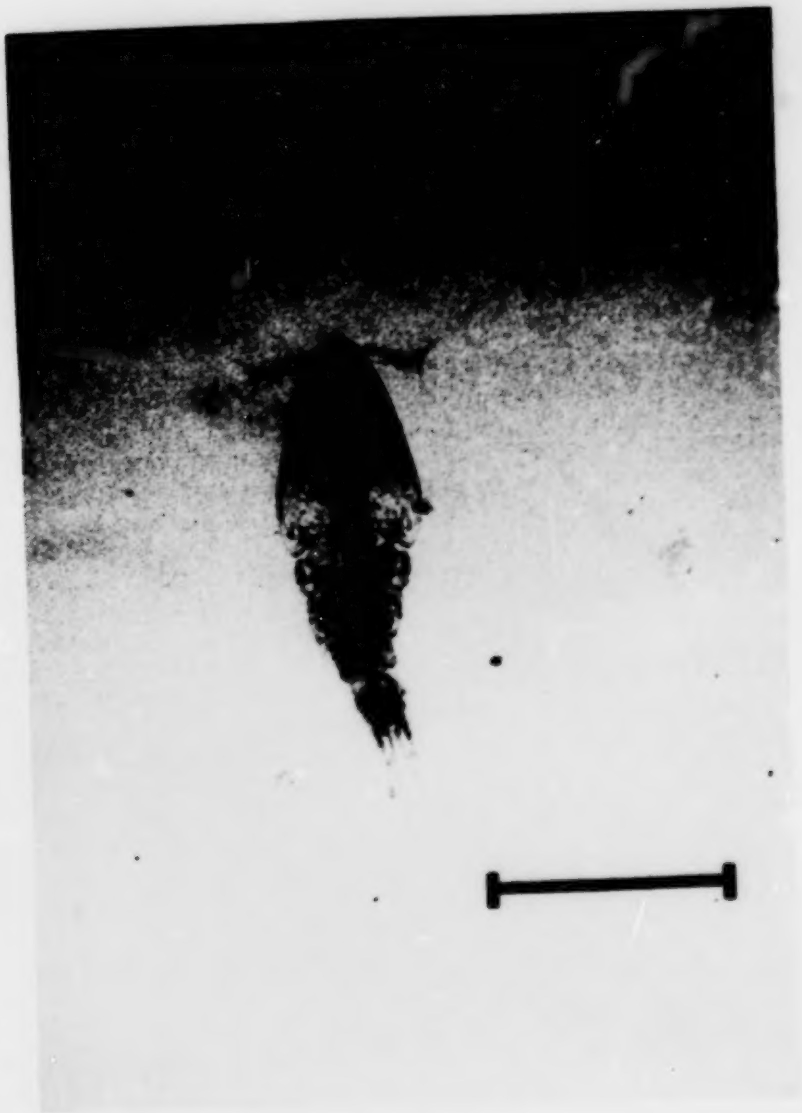

Figure IX.

Unidentified harpacticoid, an adventitious microcrustacean often found in the plankton of coastal estuaries and inlets (3.4 cm = 50μ) 685x.

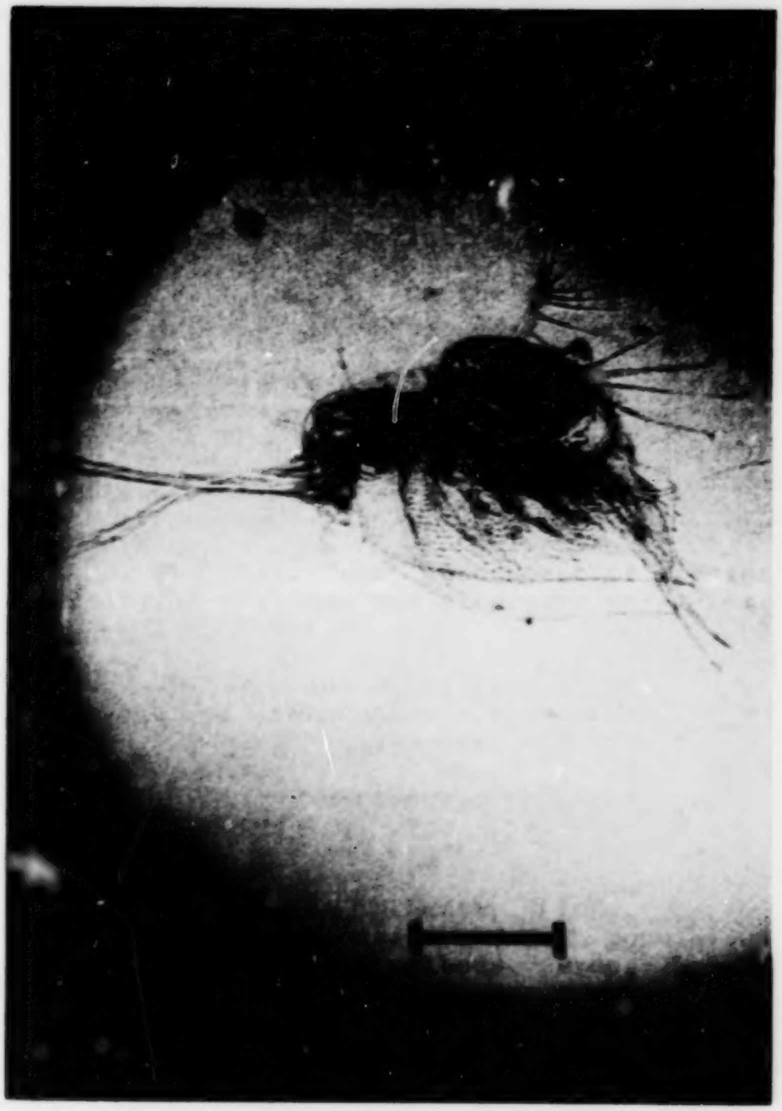

Figure X.

Penilia avirostris Dana, a marine cladoceran occasionally found in large numbers in the New York Bight. (2.0 cm = 50μ) 410x.

Conclusion

Seasonally dominate endemic zooplankton species should be used as the organisms of choice for the ocean disposal bioassay criteria, especially in light of our knowledge that these species are much more sensitive than the *Artemia* nauplii. At the present time, however, this is not feasible because the availability of these species from the laboratory stocks is extremely limited in number and kind. Field collecting could provide the organisms, but it would be unwieldly, time consuming and expensive for each laboratory performing the bioassays. Special holding facilities would be required to maintain the organisms while the bioassay was being performed.

In lieu of direct replacement of *Artemia* nauplii with seasonally dominant zooplankton species, derivations of sensitivity factors for each of the indigenous should be determined using as many of the life stages as possible. These sensitivity factors should be determined in laboratories that have the expertise and equipment to work with regionally indigenous species.

Summary

Comparative experiments using recommended bioassay procedures for *Artemia* nauplii and endemic microcrustacean zooplankton have illustrated that endemic species have a greater sensitivity to municipal sewage sludge than have *Artemia* nauplii. It has also shown, based upon these results, that the allowable concentation (0.01 of the *Artemia* 48-hour TL_{50}) may be too high to prevent impact upon the plankton in the mixing zone. In this case, the Criteria may not be sufficient to prevent damage, both lethal and sublethal, to the organisms first exposed to the dumped materials, namely the microcrustacean populations.

To increase the value of using the bioassay criteria for protecting the marine ecosystem from the impact of disposed materials, sensitivity factors must be determined for additional endemic species. These sensitivity factors coupled with the present application factors, could then be applied to the toxicity values derived from the *Artemia* bioassay to increase the use of these bioassays as a device for protecting the ecology of our Nation's coastal waters.

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MONITORING DREDGE SPOILS

L. S. Slotta and K. J. Williamson *

INTRODUCTION

The majority of U.S. waterways depend on dredging to insure adequate water depths for shipping. Other important functions of dredging include creation of land areas; mining of underwater mineral deposits; correction of erosion; and excavation of sand, gravel, shells and rocks.

Types of Dredges

Dredges can be typically categorized as either hydraulic or mechanical. Hydraulic dredges mix large volumes of water with the sediment and the fluidized slurry is pumped away as a sludge. This type of dredge results in the discharge of large volumes of water that have become in direct contact with the dredged sediments. As a result, these waters may reflect the pollutional nature of the dredge spoil.

The dredged sediments which are termed "spoils" can be disposed of by several different procedures. For hopper dredge operations, the spoils are collected in large sedimentation tanks (hoppers) aboard the dredge. These are then dumped within the estuary or offshore. For pipeline dredging operations, the sediment slurry may be pumped to a nearby diked area which is subsequently filled with the spoil.

Mechanical dredges directly resemble dry-land excavation machines and are usually mounted on a barge. This type of dredge is primarily used for projects with rocky deposits and for limited operations. Such dredges create fewer environmental concerns since interaction of the sediments with the water column is minimized. The spoils typically are barged to a land or water disposal site.

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Scope of U.S. Dredging

Dredging activities remove and redeposit tremendous quantities of material. In the U.S. in 1972, maintenance dredging and new dredging projects acccunted for the transfer of over 300 million cu. yds. and 80 million cu. yds. of dredge spoils, respectively. Total costs of these projects exceeded $150 million (2).

A soils characterization of the spoil materials of navigation channels which are maintenance dredged annually revealed that:

> "By far the largest category (approximately 153,000,000 cu. yds. per year) is that classified as mixed sand and silt. About half this value is associated with the coastal areas of the United States, and the other half the inland rivers. Approximately 30,000,000 cu. yds. per year of that category including sand, gravel, and shell is dredged from the nation's inland waterways, while the remaining 22,000,000 cu. yds. is dredged from the coastal zone. The ill-defined materials mud, clay, silt, topsoil and shale account for 80,000,000 cu. yds. per year, all but 8,400,000 cu. yds. of which are dredged from the eastern one-third of the United States. Finally, although the group including organic muck, sludge, peat, and municipal-industrial wastes accounts for only 1,400,000 cu. yds. per year, some of the more pressing environmental problems are associated with this group. Generally speaking, the materials dredged and disposed of in inland waterways are sand and gravel. The moving sand bottoms of many of the nation's navigable rivers have been a supply of sand and gravel for construction purposes for years. Again, generally, in lakes, harbors, and many areas of the coastal zones where the carrying capacity of the water is quite low, the dredged materials often consist of small, light particles such as clays and silts" (2).

Environmental Concerns

Environmental concerns in relation to dredging have risen due to the relatively fragile nature of estuarine ecosystems and the widespread use of dredging in estuaries. Particular interest has been generated around spoil disposal by filling wetlands and dumping in estuarine waters. Only the latter case and other dredging activities that could directly affect water and benthic environments are examined in this paper.

Many positive environmental impacts have been documented for dredging in addition to the obvious creation and maintenance of channels. Improved circulation which results from the removal of choked inlets can increase production of shellfish and fish due to the increased availability of food. Increased circulation also can reduce the impact of man-made wastes which are frequently discharged into estuaries. In many cases, dredge spoils are economically processed to produce sand and gravel for construction.

In contrast to the several positive impacts, many potential negative environmental impacts have been cited (Table 1). These impacts result from various physical alterations such as the change in the underwater topography, the removal of benthic animals and plants and the discharge of large quantities of particulate matter into the water column. In all cases, serious degradation of water quality and destruction of ecological systems may occur.

Table 1. Potential Negative Environmental Impacts of Dredging of Sediments

Alteration of the Estuarine Environment	Environmental Impact
Changed Topography	Alteration of Currents, Tides, Salinity Regimes, and Water Quality
Removal of Benthic Animals	Significant Animal Kills, Alteration of Important Habitats
Removal of Benthic Plants	Alteration of Pelagic and Benthic Habitats, Increased Instability of Benthic Deposits
Discharge of Particulate Matter	Increased Turbidities and Sedimentation Rates, Release of Soluble Pollutants

SCOPE

During the past fifteen months, an interdisciplinary team at Oregon State University, under the sponsorship of the NSF-RANN program, has been conducting research on the environmental effects of dredging in estuarine waters. This paper will report the potential acute and chronic environmental impacts of dredging examined as part of this study. A proposal of guidelines to minimize the acute impacts and the identification of research needs to effectively monitor dredging projects will also be presented.

ACUTE ENVIRONMENTAL IMPACTS

The interactions within estuaries are highly complex and involve geological, hydraulic, biological, chemical, social, economic and political factors. Presently, the impacts of dredging are primarily identified as acute changes in the important system properties of one or several of these categories. However, dredging also induces many potential long-term chronic impacts that also must be incorporated into the decision-making process. To fulfill national goals of protecting our environments, dredging must be regulated and both acute and chronic environmental impacts must be considered. The potential acute and chronic problems and monitoring procedures to regulate their impacts are described in the following sections.

Altered Circulation

Dredging can have a wide influence on estuarine environments by altering the circulation patterns. Many of the biological species are adversely affected by permanent changes in either salinity or temperature which result from the circulation changes. St. Amant (20) and Waldo (22) both reported long term changes in biological production to such dredge induced alterations. Some populations such as herbivore *Acartia tonsa* (10) are extremely sensitive to specific circulation patterns which can be significantly altered by either spoil removal or disposal.

These studies suggest that dredging that could significantly alter circulation patterns needs to be regulated and at least monitored. The necessary techniques of determining circulation patterns by airphoto analysis have been developed and successfully utilized (4,24).

Physical Removal of Organisms

The most apparent biological impact of dredging pertains to the removal of benthic organisms in the dredge spoil. Although this process probably does result in a large kill of these organisms, the impact does not appear to be significant for small scale dredging operations. Harrison, Lynch and Altschaeffl (8); Saila, Pratt and Polgar (14); and Slotta, et al. (19), all measured an immediate decrease in the infaunal populations after dredging, but a fairly rapid repopulation did occur.

Burial of Organisms

The ability of animals to withstand the adverse effects of burial in areas near the dredge site or in the spoil site depends primarily on their behavior and morphology. Species such as large polychaetes and bivalves which can burrow have been shown to survive burial of up to 21 cm of sediments (15). However, attached sessile species are probably killed by burial of any magnitude. Numerous authors (see Saila, Pratt and Polgar, 1971, for a review) have reported acute kills from burial of various benthic organisms including oysters. Slotta, et al. (19) reported that readjustment of benthic infauna to former abundance levels occurred within two weeks of spoiling. Thus the impacts in the spoil areas also do not seem to be significant for small localized projects.

The rapid recovery rates at both the dredge site and spoils area have been attributed to a resistant biological population (19). It has been hypothesized that the dredging-related activities, such as marine traffic, also disturb the benthic deposits, at short time intervals, which tends to ecologically favor organisms resistant to disruptions. Thus, acute biological impacts of dredging in areas subjected to dredging-related activities may not be significant even though large quantities of sediments are removed and deposited.

Turbidity and Suspended Solids

The most commonly reported effect of dredging on water quality is an increase in turbidity and suspended solids. However, some investigators have concluded that such increases do not represent a significant impact (6,11,12,14,21). This conclusion has been reached based on two premises. First, the increase in turbidity and suspended solids occurs over localized areas which pelagic species can probably avoid. Second, periodic high turbidity levels are part of the evolutionary experience of estuaries. Sediments are resuspended by wind, waves and tidal scour and large sediment loads are carried with the winter fresh water flows. Schubel (17) has reported a 20-fold increase in suspended sediment concentrations in Chesapeake Bay caused by natural occurences. With this evolutionary experience, many estuarine animals are tolerant to suspended-solids laden waters. Saila, Polgar and Rogers (15) cited several examples of tests with fish and lobsters held in water with several grams/liter of suspended sediments; no significant mortalities were measured. Thus turbidity related impacts do not seem to be significant in those cases cited.

Nutrient Release

Nutrients in the various chemical forms of nitrogen and phosphorus are commonly released from dredge spoils which results in significant increases in the ambient concentrations. Cronin, et al. (6) reported increases near the discharge plume from 50 to 1,000 times ambient total phosphorus and total nitrogen levels. However, no increase in phytoplankton was observed. Windom (24) also reported large releases of nutrients in his studies of five estuaries on the southeastern coast of the United States. However, in contrast to Cronin's results, significant algal growth was reported when dredge spoils were placed in contact with the receiving waters in closed bottle experiments. Stimulation of algal growths was also noted from light-dark bottle experiments at the dredging sites. In most cases, such factors as the localized nature of most dredging projects, the large dispersion in most estuaries and the decrease in available light from increased turbidity will reduce the potentiality of serious environmental problems from nutrient stimulation.

Oxygen Demand

Dredging operations lead to the release of organic materials and inorganic materials (such as sulfides) which create an oxygen demand within the overlying waters/ Under certain conditions, significant reductions of dissolved oxygen concentrations can result during dredging operations (3). In addition, dredging operations may expose benthic deposits of high oxygen demand which previously had been covered by relatively clean materials. The settlement of organic material suspended by dredging operations on the surfaces of benthic systems and can cause an increased benthic oxygen demand. The reverse can also be true if dredging operations lead to the removal of polluted sediments.

Free Sulphides

High concentrations of free sulfides within the deposits and the release of free sulfides to the overlying water and atmosphere as a direct or indirect result of dredging operations can be environmentally significant for a number of reasons. First, the release of free sulfides can increase the benthic oxygen demand rate and thus lead to a decline in the aerobic zone of the deposit and a rapid lowering of the DO concentrations within the overlying waters. Second, free sulfides, particularly hydrogen sulfide, are toxic at low concentrations to fish, crustaceans, polychetes, and a variety of benthic micro-vertebrates (7,9,18). Actual toxic concentrations reported in the literature usually represent only initial sulfide concentrations below 0.075 mg/l (pH 7.6-8.0) were found to be significantly harmful to rainbow trout, sucker, and walleye, particularly to the eggs and fry of these fish (5).

Heavy Metals

The release of heavy metals from polluted sediments as a result of dredging has been postulated by many authors. However, in sediments where sulfides are being produced, the possible chemical transformations from resuspension become quite complex. Presently it is unknown whether heavy metals will be released from sulfide bearing sediments.

Ferrous sulfides are common minerals in anaerobic sediments and are probably responsible for the characteristic black color. Preliminary studies at OSU have shown that heavy metals absorb on both Fe(III) oxides and Fe(II) sulfides. In addition, the heavy metals are readily co-precipitated in both Fe(III)oxides and Fe(II) sulfides. From these results, it is hypothesized that heavy metals will not be released to the water column upon resuspension and either will be absorbed, co-precipitated and incorporated within the sulfide-bearing sediments; a similar hypothesis has been proposed by Windom (24). The hypothesis is in agreement with the data reported by Windom (24) and May (12) in which heavy metals present in the dredge spoils were not released to the water column.

More research is required to elucidate the important mechanism occurring in this process. Present data are not adequate to establish exact criteria.

Toxic Organics

Important organics in relation to the toxicity of dredge spoil include the organochloride insecticides, the organophosphorus insecticides and the polychlorinated biphenols. The possible adverse effects of spoils contaminated with these compounds are numerous; however, direct cause and effect relationships are not well documented. More research is needed in this area in relation to monitoring methods and acceptable criteria.

CHRONIC ENVIRONMENTAL IMPACTS

As described in the last section, the acute impacts of dredging are highly complex and not well-defined. Even less is known about the extent of chronic or long term environmental impacts. These chronic impacts include not only dredging but also such activities as shipping, industrialization, and urbanization which alter the environment in complex ways. The measurement of such chronic impacts requires an understanding of the geological, hydraulic, biological and chemical factors which control the interactions in estuaries.

Presently, the impacts of dredging have been primarily identified as acute changes in important system properties. Little is known of chronic impacts for two reasons. First, chronic impacts are not so immediately apparent upon examination of a problem. An understanding of the system properties is often required to sort out the chronic problem from the multitude of other changes. In reference to dredging, the understanding of important system properties has been almost non-existent. Second, the detection of chronic impacts requires long-term research. Several potential chronic impacts will be briefly discussed in this section.

Particle Size Change

A dominant feature of hopper dredging activities is the resuspensions of bottom sediments. As a dredge suction head passes through a dredge site, surface sediments drawn into the head and pass to the hopper. Some of the material around the suction head is disturbed mechanically and thrown into suspension. Heavier particles settle out after the disturbance passes, while lighter particles remain in suspension due to ambient turbulence and may be transported from the original site by local currents. The material which passes into the hopper is initially in suspension, but the heavier particles settle to the hopper bottom. The lighter particles remain in suspension and some are returned to the estuary water column via the hopper overflow.

At the spoil area, the contents of the hopper are released and settle to the bottom as a slurry. Surface shear during descent and impact-induced mixing at the bottom resuspend a portion of the material; again, the fines may be transported from the spoil site. As a result of repeated resuspensioning and settling and the subsequent loss of fines, dredge spoils may contain smaller fractions of fines than occur at the dredge site.

Specifically, it was observed on the Coos Bay hopper dredge project that a five-fold increase in mean particle size occurred in the spoils immediately after spoiling and persisted for two months. The escaping fines probably contributed to long term siltation in the adjacent shallow areas.

The dependence of animal populations on a specific particle size distribution has been clearly identified. Rhoads and Young (13) reported that suspension feeders and benthic infauna are largely confined to sandy or firm mud bottoms. Sanders (16) showed that suspension feeders in Long Island Sound comprised 80 percent of the organisms on coarser sediments. Selective and non-selective deposit feeders were the dominant forms in fine sediments. Thus it can be concluded that changes in particle size from dredging operations probably seriously affect the distribution of the benthic populations.

Reduced Sediment Turnover

Polluted dredge spoils are often deposited behind "water tight berms" and/or in diked, sacrificial channels. In either case, a sediment system results in the spoil deposit area in which the bottom deposits have increased stability over their previous condition. With this increased stability the sediments are turned over less frequently and the build-up of anoxic, sulfide-bearing sediments can result. In addition, more organics are deposited in this relatively quiescent region which further encourages the growth of sulfate-reducing bacteria. The end result in the spoil disposal area can be s significant reduction in the biological populations present before spoiling. Similar results can occur in open estuarine areas when dredging causes reduced current velocities throughout an estuary.

Increased Sediment Turnover

Dredging can increase current velocities through the removal of inlet barriers, channelization, or the removal of eel grass. These increased current velocities may subsequently increase the turnover of the sediments, which, as the reverse of the previous case, can also adversely affect the biological communities.

Resistant Biological Communities

Preliminary studies (19) have suggested that the benthic infaunal communities may become modified in an estuary which has repeated dredging. These communities may become adapted to a more or less continual resuspension of the sediments and its persistence may actually depend on this turnover. The turnover in some estuaries may depend more upon the prop wash of large ships than on the continual maintenance dredging.

RESEARCH NEEDS

In relation to monitoring of dredging projects the following research areas should be addressed.

Improve Monitoring Requirements

A system needs to be developed in which the required parameters to be monitored varies with the degree of pollution. Some easily measured parameters (e.g., volatile solids) that roughly correlate with pollution potential should be used to determine both the sampling methods and the required parameter to be monitored. For low volatile solids (<2% by dry wt.), little additional monitoring would be necessary; for high volatile solids (>10% by dry wt.) many tests both before and during the dredging would be necessary.

Release of Heavy Metals and Toxic Hydrocarbons

Research needs to be initiated to determine if heavy metals can be released from dredge spoils under natural environmental conditions. Additional studies are required to elucidate the important transport mechanisms and the environmental impacts of the chlorinated hydrocarbons which are known to exist in high concentrations in certain sediments.

Turbidity

The exact role of turbidity as a controlling factor is relatively unknown for estuaries. Work concerning long term increases from all man-made activities including dredging and the possible impacts of such increases should be established.

Turnover of Benthic Deposits

Man-made activities in estuaries undoubtedly alter the rate at which benthic deposits are turned over. Natural causes include tides, currents, freshwater flows and benthic burrowers. Important man-related causes are dredging, ship props, and channelization. The interrelationship and importance of each of these activities needs to be further examined.

CONCLUSIONS

1. Present monitoring technology is available to measure many parameters in relation to dredging projects.
2. Criteria are required to specify which parameters should be monitored.
3. More research is necessary to elucidate cause and effect relationships especially in relation to chronic impacts.

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COMPARISON OF SPECIES DIVERSITY AND FAUNAL HOMOGENEITY INDICIES AS CRITERIA OF CHANGE IN BIOLOGICAL COMMUNITIES

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INTRODUCTION

The structure of marine communities is frequently used as an indication of the environmental consequences of pollution (21). Prevention of major ecosystem alterations necessitates monitoring programs that can detect subtle changes before irreversible damage has occurred. Data analysis procedures should therefore be sensitive to relatively minor differences in biological conditions. Species diversity indices have become very popular criteria (sometimes the only criterion) of the "stability" or condition of stressed ecosystems (1, 2, 5, 10, 13, 20, 24, 25, 26, 27). Other community characteristics such as species composition, homogeneity, density, and population dynamics are often ignored. In this report we will compare the ability of a variety of structural indices to discriminate between closely related assemblages of demersal fishes and macroinvertebrates at four stations in Yaquina Bay, Oregon.

MATERIALS AND METHODS

Data Collection

Demersal fish and epibenthic crustaceans were collected by bottom trawl at four stations in the Yaquina Bay, Oregon from March 1967 through Febraury 1968. Two surveys, usually separated by a two-week period, were made each month. Data from each station have been pooled on a monthly basis for analysis. The stations were located from near the Yaquina Bay entrance (station 1) to Weiser Point, 7.6 km up the Bay (Table 1). Collections were made with a semi-balloon 5.9 m (headrope length)) trawl net with a 3.8 cm body mesh and 1.3 cm (stretched size) bag liner. All specimens were identified to the species level and enumerated.

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Data Analysis

Density

Because of differences in trawling distances at the four stations, densities were compared on the basis of catch of individuals per unit effort. The minimum effort was a 550 m total monthly trawl distance at station 3. Faunal densities for stations 1, 2 and 4 were calculated by multiplying the observed catch by the ratio of 550 m to the actual monthly trawl distance at each station.

Table 1. Description of trawl stations.

Station Number	Location	Distance from entrance to jetties at Newport, Oregon	Approximate trawl distance
1	First rock finger downbay from Hwy. 101 bridge to south bridge support.	1.4-1.8 km	400m
2	Parallel to Newport breakwater.	2.6-3.4 km	750m
3	Buoy 15 to opposite Conquille Point.	5.7-6.0 km	275m
4	Between Yaquina and Weiser Points.	7.0-7.6	560m

Species diversity

Species diversity is dependent on the number of species (richness) and the distribution of individuals among the species (evenness) (16). Shannon's (19) information-theoretical measure of mean species diversity per individual (H') is sensitive to and increases with both species richness and evenness. The value of H' is proportional to the uncertainity of identification of an individual selected at random from a multi-species population (16). H' was calculated with the aid of the tables of Lloyd, Zar, and Karr (1968) according to:

$$H' \approx \frac{1}{N} \left(N \log_{10} N - \sum_{i=1}^{S} n \log_{10} n_i\right)$$

where n is the number of individuals of the $i^{\underline{th}}$ species, N is the total number of individuals, and S is the total number of species.

The evenness of each collection was determined a J', the ratio of observed H' to maximum H' for observed S(16). Maximum H' occurs when all species are represented by the same number of individuals. Thus for H' max:

$$\sum_{i=1}^{S} n_i \log_{10} n_i = S\left(\frac{N}{S} \log_{10} \frac{N}{S}\right)$$

and the equation for H' reduces to:

$$H' \max = \log_{10} S$$

The evenness index $J' = H'/H' \max = H'/\log_{10} S$

Indices of species richness were determined in three ways:

1. the observed number of species (S) in each monthly collection

2. the predicted number of species per unit effort (S_R). Sanders' (18) rarefaction method was used to predict the number of species that would have been present if the trawling distance had been 550 m per month at all stations. All n /N were calculated for the original sample. The percentage that one individual would represent in the 550 m trawl ($1/N_{550}$) was determined from the faunal density

data. It was assumed that all species for which $(n_i/N) \gg (1/N_{550})$ would be present in the rarefied sample. In addition, some of the rarer species for which $(n_i/N) < (1/N_{550})$ would also be present. Their contribution to S_R was determined by $(n_i/N)(N_{550})$.

3. Margalef's (1951) species richness index:

$$d = (S-1)/\log_e N$$

Faunal affinity

An affinity index (17) was used to document faunal homogeneity between pairs of monthly collections. The percent of the total abundance represented by each species in each sample was calculated. The index value was obtained by summing the lower percentage for all species common to both samples. For example, if species X represented 10% of sample A and 20% of sample B, 10% of the total affinity between the samples would be attributed to species X. If all species were common to both samples and if the distribution of individuals among the species was the same on a percentage basis, the index value would 100%. If there were no species common to both samples, the index value would be 0%.

RESULTS

Density

Catch per unit effort was highest at all stations from May through August (Fig. 1). Although the seasonal pattern of change was the same, there were substantial differences in absolute densities at the four stations. The maximum monthly catch of individuals was 1360 in June at station 3; and 1090, 230, and 160 in July at stations 4, 2, and 1, respectively. Mean monthly catches from May through August were 123, 146, 1142 and 621 at stations 1 through 4, respectively. During the remainder of the year (September through April) the mean catches were 48, 42, 147, and 53 at 1 through 4, respectively. Thus the density of the demersal macrofauna was highest throughout the year at station 3, relatively high during the summer at station 4, and relatively low and very similar at stations 1 and 2 during the entire year.

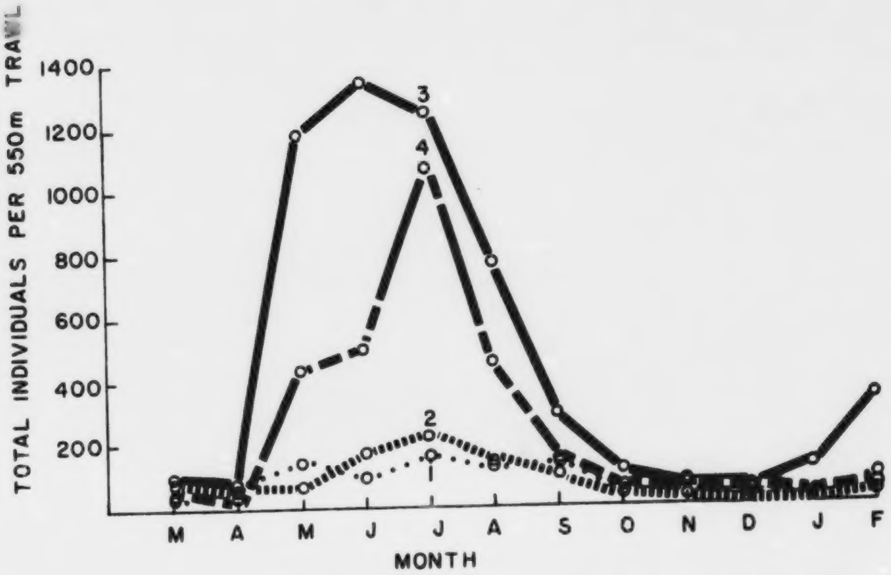

Fig. 1. Seasonal changes in faunal density (catch per 550 m trawl distance) at stations 1-4.

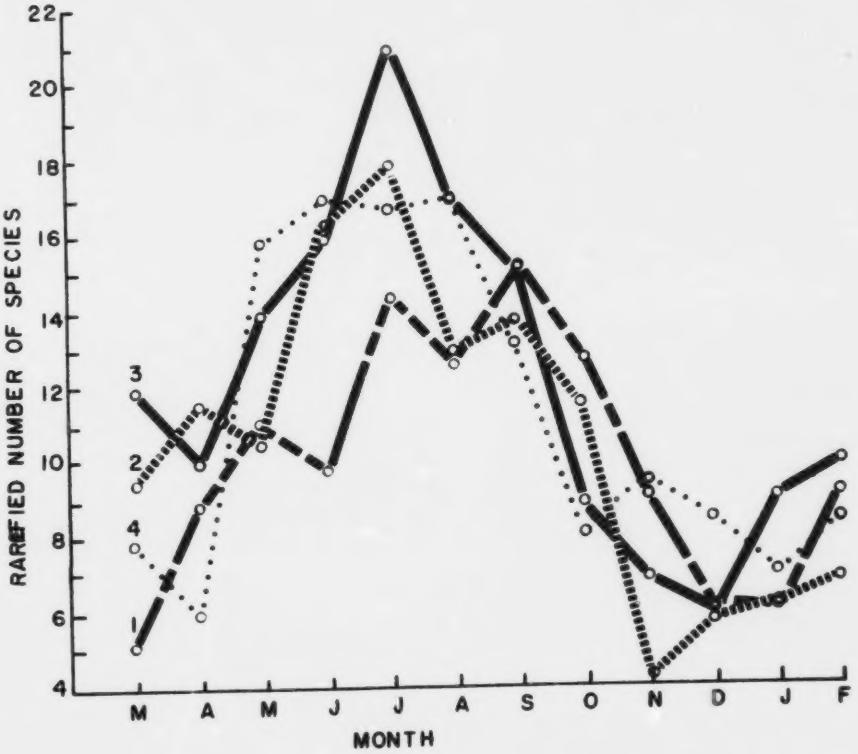

Fig. 2. Seasonal changes in species richness (S_R) per 550 m monthly trawl collection at stations 1-4.

Species diversity

Species richness

The spatial-temporal distribution patterns of S (observed number of species) and S_R (rarefied number of species per 550 m monthly trawl) were essentially identical. Only the latter will be presented. S_R increased during the summer at all stations and, compared to density, there were relatively small differences in species richness between stations (Fig. 2). The maximum S_R at each station was highest at 3 (21 species) and lowest at 1 (15.1 species), although during two months (September and October) S_R at 1 was higher than at any other station. There was very little difference in species richness when data for the entire year were pooled: S_R = 28.8, 28.0, 28.0, and 29.9 for stations 1 through 4, respectively.

Margalef's (8) richness index, d, was also generally higher during the summer, but its seasonal pattern was not as obvious as that of S_R (Fig. 3). Since d is a function of the ratio of richness to density [$(S-1)/\ln N$], the index value was often lowest at station 3 where density was usually 2 to 3 times greater than at the other stations. The highest d occurred at station 1 for the October collection which contained 16 species and 67 individuals.

Evenness

There was little evidence for a seasonal cycle in Pielou's (16) evenness index (J') at stations 1 and 2 (Fig. 4). J' at stations 3 and 4 was very low from May through July and it was consistently lower than J' at 1 and 2 during late spring and the entire summer. During the remainder of the year, there were no clear differences in J' at the four stations.

Information-theoretical index of species diversity

Shannon's (19) index (H') increased during the warmer months at stations 1 and 2 (Fig. 5), but the seasonal pattern was not as obvious as that of species richness. H' was lower at stations 3 and 4 than at 1 and 2 from late spring to early fall. Like J', H' at 3 and 4 was relatively low during the months of highest faunal densities (May-July).

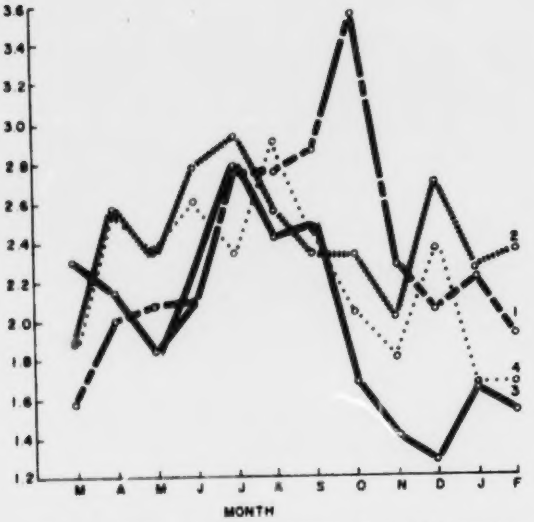

Fig. 3. Seasonal changes in Margalef's (1951) species richness index (d) at stations 1-4.

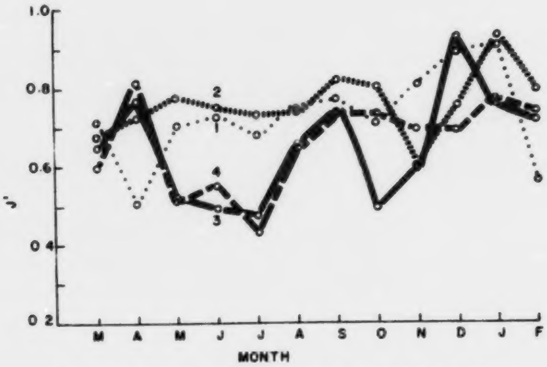

Fig. 4. Seasonal changes in Pielou's (1970) evenness index (J') at stations 1-4.

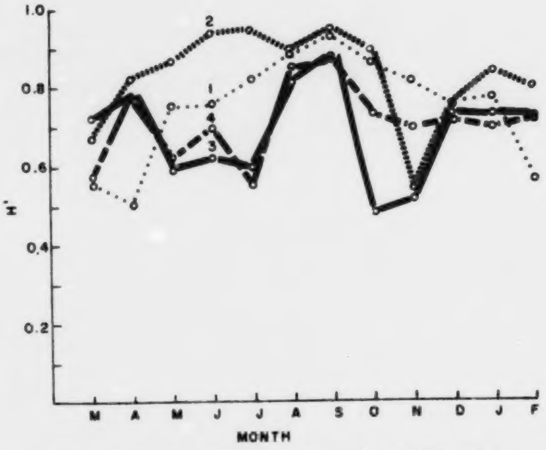

Fig. 5. Seasonal changes in Shannon's (1948) species diversity index (H') at stations 1-4.

Faunal homogeneity

Except for September, the faunal affinity index between monthly samples at stations 1 and 2 (annual mean ± $S_{\bar{x}}$* $t_{.05}$ = 40.0 ± 7.6) was consistently higher than between 1 and 3 ($\bar{x}$ = 19.6 ± 6.1) or 1 and 4 ($\bar{x}$ = 17.5 ± 6.4) (Fig. 6). The assemblage at station 2, however, was just as closely related to stations 3 ($\bar{x}$ = 44.2 ± 8.4) and 4 ($\bar{x}$ = 39.3 ± 7.4) as 1 (Fig. 7). Highest faunal homogeneity occurred between stations 3 and 4 ($\bar{x}$ = 68.9 ± 7.9) (Figs. 8 and 9). Thus, the analyses of faunal homogeneity indicated the existence of two distinct assemblages at stations 3-4 and 1, and an intermediate demersal community at station 2. This pattern was evident throughout the year.

Spatial-temporal distribution of species

Enophrys bison

The buffalo sculpin was the most abundant species at station 1, and second most abundant at 2 (Fig. 10). It ranked 14th at 3 and 15th at 4. Thus its spatial distribution accounted for much of the relatively high affinity between stations 1 and 2 and low affinity between 1-3 and 1-4. Most of the specimens in our collections were juveniles < 10 cm in total length. They were most abundant from April through October.

Cancer magister

The dungeness crab was the most dominant species at station 2 and more common at 3 and 4 than at 1 (Fig. 11). Juvenile crabs enter Yaquina Bay in large numbers during June and July and most of the population returns to the ocean in the fall.

Parophrys vetulus

The English sole was the most numerous species at stations 3 and 4 (Fig. 12). It ranked 3rd and 7th in abundance for the entire year at stations 2 and 1, respectively. Only a few specimens in our samples and none in those of Westrheim (22) from the same area were adults. Although present throughout the year, *P. vetulus* was most common from May through August.

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* $S_{\bar{x}}$ = standard error

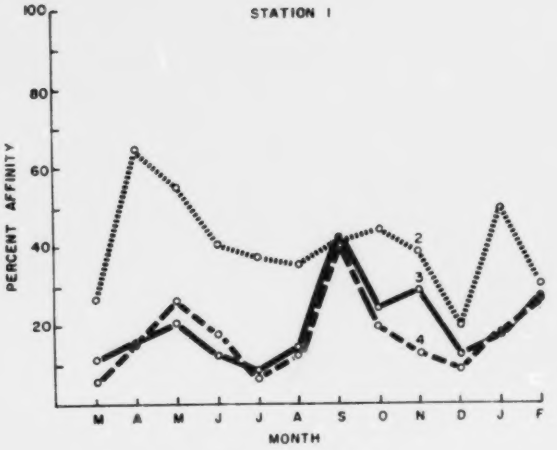

Fig. 6. Faunal affinity between station 1 and stations 2, 3, and 4.

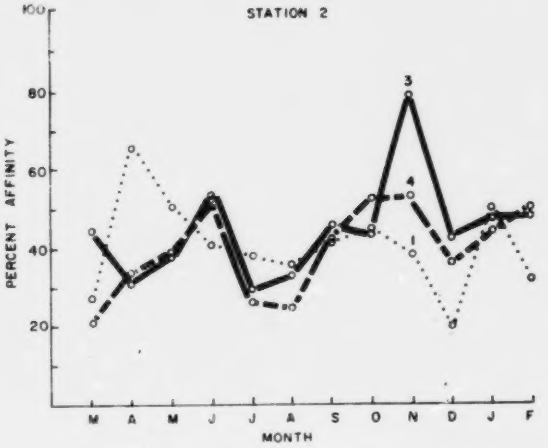

Fig 7. Faunal affinity between station 2 and stations 1, 3, and 4.

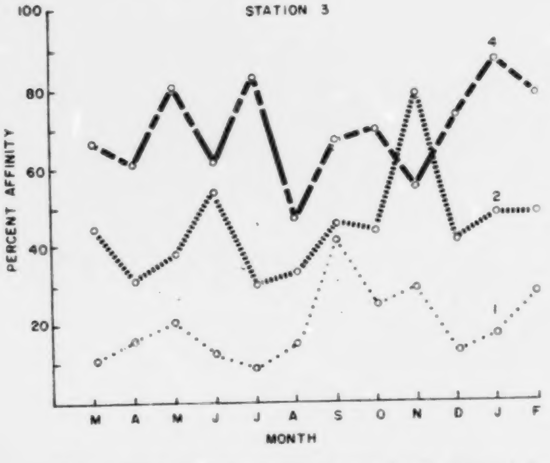

Fig. 8. Faunal affinity between station 3 and stations 1, 2, and 4.

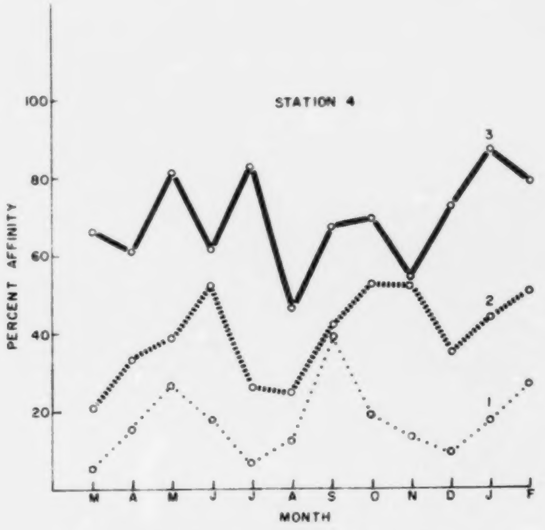

Fig 9. Faunal affinity between station 4 and stations 1, 2, and 3.

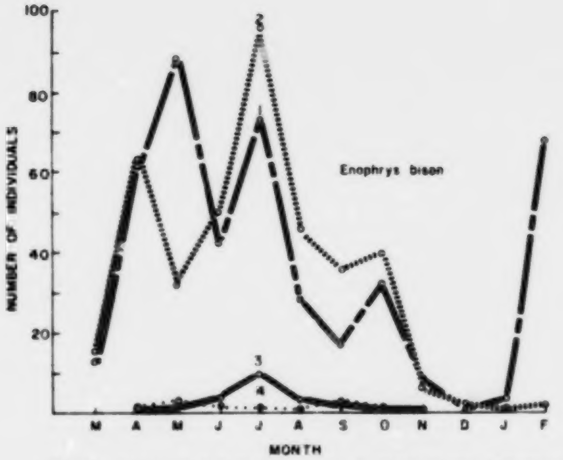

Fig. 10. Seasonal distribution of the buffalo sculpin (Enophrys bison) at stations 1-4

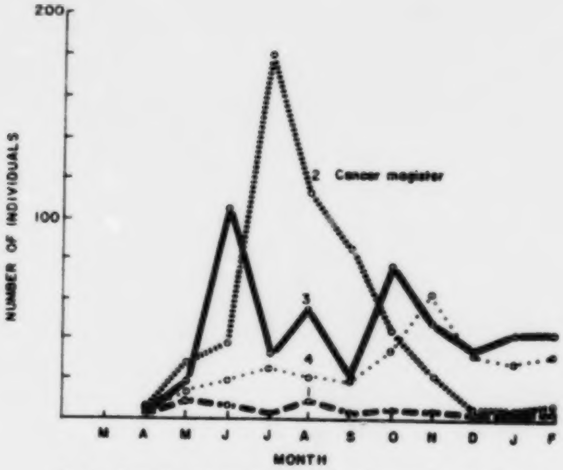

Fig. 11. Seasonal distribution of the Dungeness crab (Cancer magister) at stations 1-4.

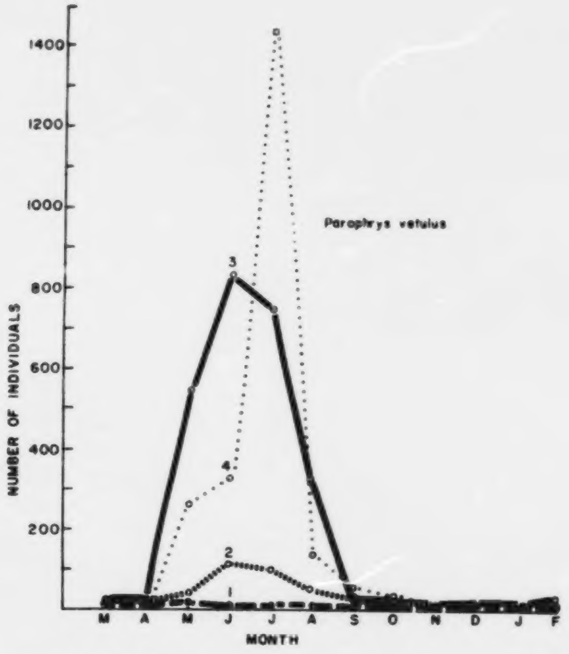

Fig. 12. Seasonal distribution of the English sole (Parophrys vetulus) at stations 1-4.

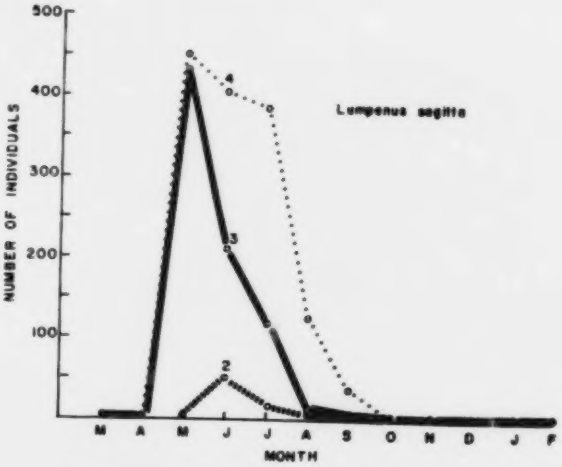

Fig. 13. Seasonal distribution of the Pacific snakeblenny (Lumpenus sagitta) at stations 1-4.

Lumpenus sagitta

The Pacific snakeblenny was the second most abundant species at stations 3 and 4, ranked eighth at station 2, and was not collected at station 1 (Fig. 13). Like Parophrys vetulus, L. sagitta was very abundant in the late spring and early summer. From May to August these two species represented 70% of the individuals collected at stations 3 and 4.

Platichthys stellatus

The starry flounder was common throughout the survey area. It ranked 4th, 6th, 5th, and 6th in total abundance at stations 1 through 4, respectively (Fig. 14). It was least abundant in the fall, but was present in more monthly collections (45 of 48) than any other species.

Rhacochilus vacca

Mature specimens of the viviparous pile perch entered Yaquina Bay during the spring. Many young-of-the-year were present at station 4 in August and at lower Bay stations in September (Fig. 15). The spatial-temporal distribution of R. vacca contributed to several deviations from the normal pattern of faunal affinity between the stations. The minimum index value between stations 3 and 4 occurred in August when R. vacca was abundant at 4 and rare at 3. Maximum affinity between stations 1-3 and 1-4 was observed in September when pile perch were common at all stations.

Crago nigricauda

The black-tailed shrimp is of interest because of its unique seasonal distribution (Fig. 16). It was the only species common from late fall to early spring and rare or absent during the summer.

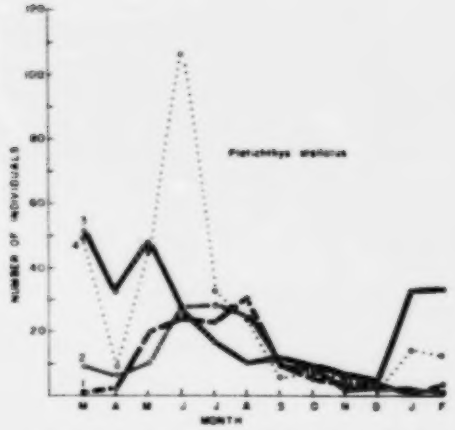

Fig 14 Seasonal distribution of the starry flounder (*Platichthys stellatus*) at stations 1-4

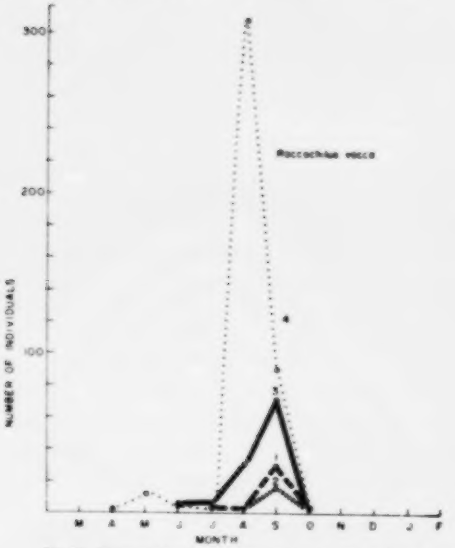

Fig 15 Seasonal distribution of the pile perch (*Roccochilus vacca*) at stations 1-4

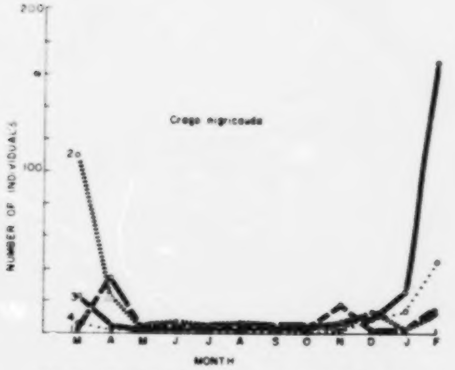

Fig 16 Seasonal distribution of the black-tailed shrimp (*Crago nigricauda*) at stations 1-4

DISCUSSION

Spatial-temporal changes in the structure of the demersal community of Yaquina Bay are complex. No single index adequately reflected all of the differences between the four stations. In fact, the interpretation of some indices may have been misleading in the absence of other community and population characteristics.

The density of all individuals was relatively high in the upper bay (stations 3 and 4) and low near the bay entrance (stations 1 and 2). This difference was due primarily to the very high abundance of just two species (*Parophrys vetulus* and *Lumpenus sagitta*) at stations 3 and 4, and was not characteristic of the rest of the assemblage. The extreme dominance of *P. vetulus* and *L. sagitta* explains the reduction in J' at the upper bay stations from May to August.

Species richness (S_R) was very similar at all stations but the faunal affinity index demonstrated major changes in either species composition or dominance. Margalef's index, d, was influenced by both richness and density patterns. These parameters are best analyzed separately as S_R and catch per unit effort or space.

H', the most popular diversity index in pollution studies, showed very little difference between the stations. Its value is a function of species richness and evenness, but it did not indicate the strong seasonal cycle of S at all stations nor the obvious difference in evenness between stations 1-2 and 3-4 from May through August. Species diversity indices derived from information theory (H' and J') are not sensitive to changes in species composition or the dominance rank of species provided that S and evenness do not change. Obviously H' should not be the sole or even primary criterion of biological conditions in marine ecosystems which often exhibit strong spatial-temporal gradients of species composition.

Neither density nor any of the species diversity indices evidenced the consistent differences in faunal homogeneity between the stations. The affinity index demonstrated the close relationship between the assemblages at stations 3 and 4, the intermediate nature of the fauna at station 2, and the dissimilarity between the communities at station 1 and stations 3-4. This simple index can also be used to document temporal changes in community structure. For example, at station 3 the affinity index was 79.8% for the

June and July collections and 15.7% for the June and December collections. This analysis is sensitive to evenness, species composition, and the relative abundance of individual species. It is not a good measure of richness because rare species enter or leave the community with little impact on the percent distribution of individuals between species. It will also not indicate differences in the density of the total community.

An analysis of the dynamics of individual populations was an essential part of the description of the demersal community of Yaquina Bay. The spatial and temporal distributions of species revealed no discrete community types with characteristic species composition and dominance. The concept of gradient analysis in which species are considered to be distributed independently according to their own environmental requirements is a more appropriate basis for description (23). Thus, some of the species (e.g. *Platichthys stellatus*) were common at all of the stations, while others occurred in maximum abundance at discrete positions of the gradient: *Enophyrys bison* at 1 and 2, *Cancer magister* at 2, *Lumpenus sagitta* and *Parophrys vetulus* at 3 and 4. The dominant organisms showed strong seasonal distribution patterns associated with migratory (e.g. *Cancer magister*) or reproductive (e.g. *Racochilus vacca*) cycles. Many populations were most abundant during the warmer months (not always the same month), but *Crago nigricauda* was least common during the summer. The independent spatial-temporal distribution patterns of single populations explain many of the apparently erratic changes in "community" characteristics. As noted above, the appearance of large numbers of young-of-the-year *Rhacochilus vacca* at station 4 in August and their subsequent migration down and out of the Bay in September resulted in deviations from the otherwise consistent pattern of faunal homogeneity between stations.

A multitude of faunal density, diversity, richness, evenness, and affinity indices have been proposed and compared on the basis of ecological and mathematical theory and empirical observations of their ability to discriminate between samples (3, 4, 6, 9, 11, 12, 13, 14, 15, 16, 18, 24). Many of these reports attempted to identify the "best" index. The objective of most marine monitoring programs conducted by regulatory agencies is to document changes in biological conditions that can be correlated with water quality or other pollution parameters. Our study has demonstrated that many characteristics of the biota should be examined. No single index is sufficient. We found that total faunal density, species richness (18), evenness (J'; 16), and an affinity index (17) were helpful in describing closely related demersal communities. Differences in the spatial-temporal distribution of individual species populations provided the most obvious discrimination between collections.

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SAMPLING METHODS FOR MICROBIOLOGICAL ANALYSES

R. R. Colwell*

Since the marine environment encompasses the coastal, off-shore and deep ocean regions of the world oceans, sampling problems cannot be truly appreciated without some knowledge of the underwater terrain, i.e., that unique topography and geography of the marine environment which may include valleys, plains, peaks, ridges, shelves and sills submerged to depths from a meter to several miles. The water overlying this vast ocean territory disguises the great heterogeneity of the deep ocean. Too often, investigators are oblivious to the changing seascape as they move from sampling station to sampling station. The geology of each region of the world oceans can be vastly different. For example, sediments may be composed predominately of sand, silt, gravel or other soil types. Replicability of samples adds another dimension to the problem. These factors pose severe problems in obtaining representative samples preliminary to describing the microbiology of a given sector of the marine environment.

A sampling regimen undertaken in a monitoring program will, of necessity, involve ship time, with the sophistication of the vessel ranging from a small "whaler" to an ocean-going oceanographic vessel. The work undertaken, therefore, is further limited by the facilities of the vessel out of which the investigator must operate. A monitoring program aboard ship can involve highly sophisticated procedures or extremely primitive or simplistic techniques.

In our studies, we have had available a range of vessels, including the R/V EASTWARD (Fig. 1) for deep ocean work. The R/V EASTWARD offers laboratory facilities permitting relatively complicated experiments at sea. Another vessel, the R/V RIDGELY WARFIELD is employed in our work in the Chesapeake Bay (Fig. 2). Laboratory space on the R/V WARFIELD is sufficient and well-designed so that several investigators can operate simultaneously and in reasonable comfort (Fig. 3).

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Figure 1. The R/V EASTWARD, operated out of Beaufort, North Carolina, by Duke University with support from the National Science Foundation.

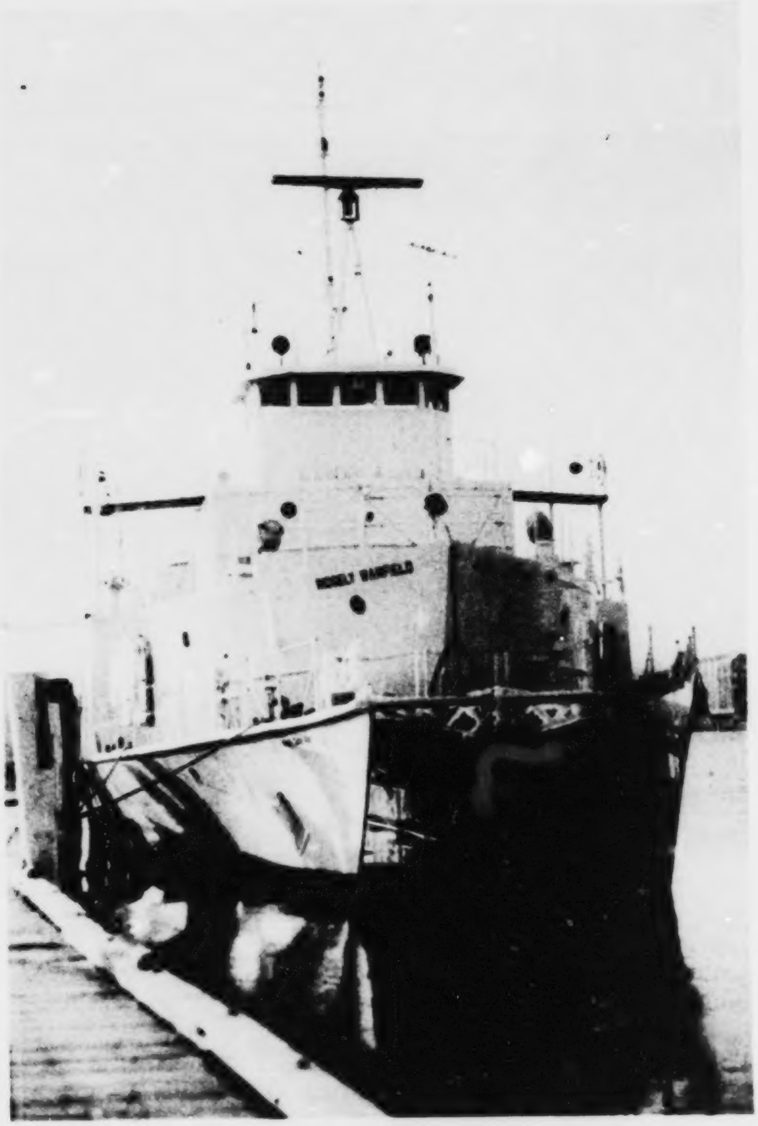


Figure 2. The R/V RIDGELY WARFIELD, a catamaran operated by the Chesapeake Bay Institute, Johns Hopkins University, with support from the National Science Foundation.

Figure 3. Laboratory aboard the R/V RIDGELY WARFIELD. Microbiological analyses can be carried out with relative ease aboard the ship.

For shallow water work, however, the size of the craft is necessarily much smaller, and on-station work thereby is significantly reduced. The Maryland Department of Natural Resources and the Environmental Protection Agency, Annapolis Laboratory, operate small craft suitable for estuarine work (Figs. 4 and 5). For very shallow streams and inlets, motor "whalers" or row-boats are used; in such craft it is feasible only to collect samples that are then returned to the laboratory for analysis. If sampling is done relatively close to the laboratory, little or no problem in delay of sample processing is encountered. If samples must be collected at great distances (more than several hours journey) from the home laboratory, either the samples are flown back to the laboratory or a mobile field laboratory unit is required.

The sampling equipment employed in collecting water, sediment and biota for microbiological analyses is relatively primitive. Marine microbiology research and monitoring are seriously hampered by the lack of proper equipment for sampling and *in situ* study. There has been a much too limited effort to improve the technology for environmental assessment and analysis. Clearly, sampling equipment for microbiological work is a problem area and deserves attention.

Determination of microbial densities can be accomplished by direct microscopic or total viable count methods, measurement of adenosine triphosphate (ATP), diaminopimelic acid (DAP), muramic acid or deoxyribonucleic acid (DNA), or the estimation of the production of heterotrophic bacteria using C^{14} uptake, measurement of oxygen consumption, bacterial photosynthesis, or bacterial chemosynthesis. For these determinations, water and sediment samples are usually collected in glass or clear plastic bottles.

Microbiological research places heavy demands on sampling techniques. The microbiological sampler has to be sterilizable. This eliminates the use of the Nansen (Fig. 6a), van Dorn and similar non-sterilizable bottles (Fig. 6b). Equipment must be available for lowering the collecting vessel to the desired depth and for its opening, filling, and closing by a triggering mechanism. The different types of microbiological sampling vessels are (a) evacuated bottles, (b) rubber bulbs, (c) plastic bags and (d) syringes.

Sorokin (11) has designed a simple bottle sampler for sampling to a depth of 50 or 60 meters. It consists of an evacuated sterile bottle fastened to a heavy frame. In

Figure 4. Maryland Department of Natural Resources craft made available for field work.

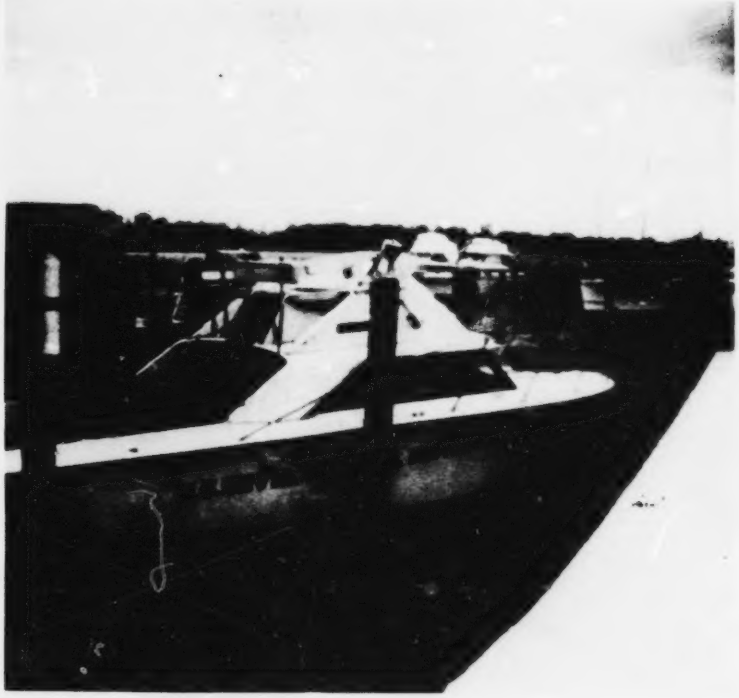


Figure 5. Research craft operated by the Environmental Protection Agency, Annapolis Laboratory. Courtesy of Dr. D. Lear.

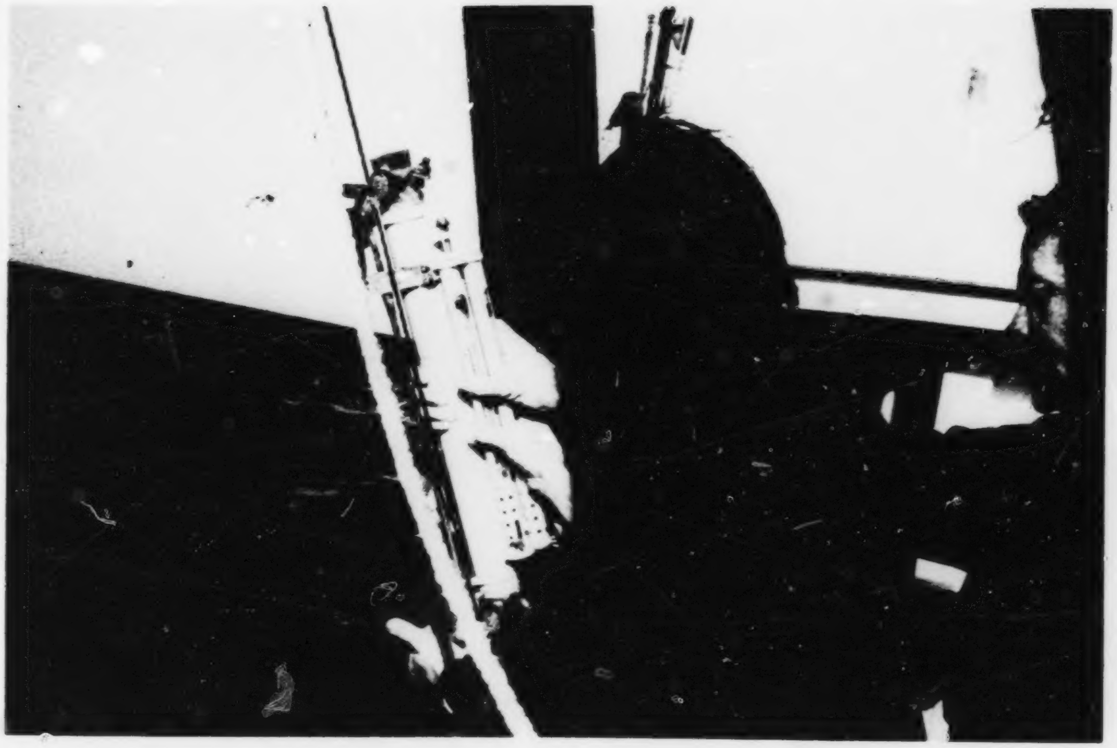


Figure 6a. The Nansen Bottle.

6b. The van Dorn Bottle. These sampling bottles, used by biological and chemical oceanographers, are unsuitable for microbiological analyses.

shallow waters, from 1 - 5 feet, many investigators choose the expediency of a sterile reagent bottle attached to a string or rope (Fig. 7). Thus, there is a need for a standardized, lightweight, hand operated, sterilizable water sampler which permits taking a sample aseptically, of known volume, and at a known depth in shallow waters.

Rubber bulbs of approximately 300 ml volume were introduced as sampling vessels by ZoBell (13) and Sieburth (10) (Fig. 8). They can be autoclaved and evacuated by deflation and used to a depth of several hundred meters.

When non-elastic plastic material is used for a sampling vessel, filling must be operated by an external source of power. This is realized in the Niskin sampler (9). Upon triggering by a messenger, two plates open in V-fashion by spring power, thus inflating a plastic bag. The intake tube of silicone rubber is cut by a sharp blade and, after filling of the bag, is closed by a clamp. There are 1.2, 3 liter and 5 liter models of the sampler (Fig. 9).

In the bulb and bag samplers, contamination of the water sample may result from breaking the glass capillary or from cutting the rubber tube intake by a non-sterile object. Furthermore, the samples are taken near the sampling gear, and the hydro-wire can be heavily contaminated by passing through the neuston layer. Escherichia coli, contaminating the surrounding surface water of a research vessel, has been shown by Jannasch (3) to be carried down to great depths by the sampling gear. To prevent such contamination, a special sampling device was constructed and tested by Jannasch and Maddux (4). This device consists of a plastic syringe operated by gravity. The sample intake is a tube of silicone rubber 10 cm long which, before autoclaving, is covered by a piece of dialysis tubing. In operation, the sampler swings about 60 cm away from the hydro-wire and a plunger sucks the sample into the syringe. Experiments showed that heavy artificial contamination of the sampling gear with a tracer organism did not result in contamination of the sample. The sampling vessel contains no air and can be operated at any depth. However, only 50 ml of water can be sampled. Thus, the application of such a sampler, in its present configuration, is limited. There is no reason why a larger volume sampler could not be designed and developed to operate on this principle.

Multiple sampling, i.e., many individual samples at a given depth cannot be obtained at the present time. That is, samples cannot be replicated except by raising and lowering a hydrographic wire outfitted with fresh sterile

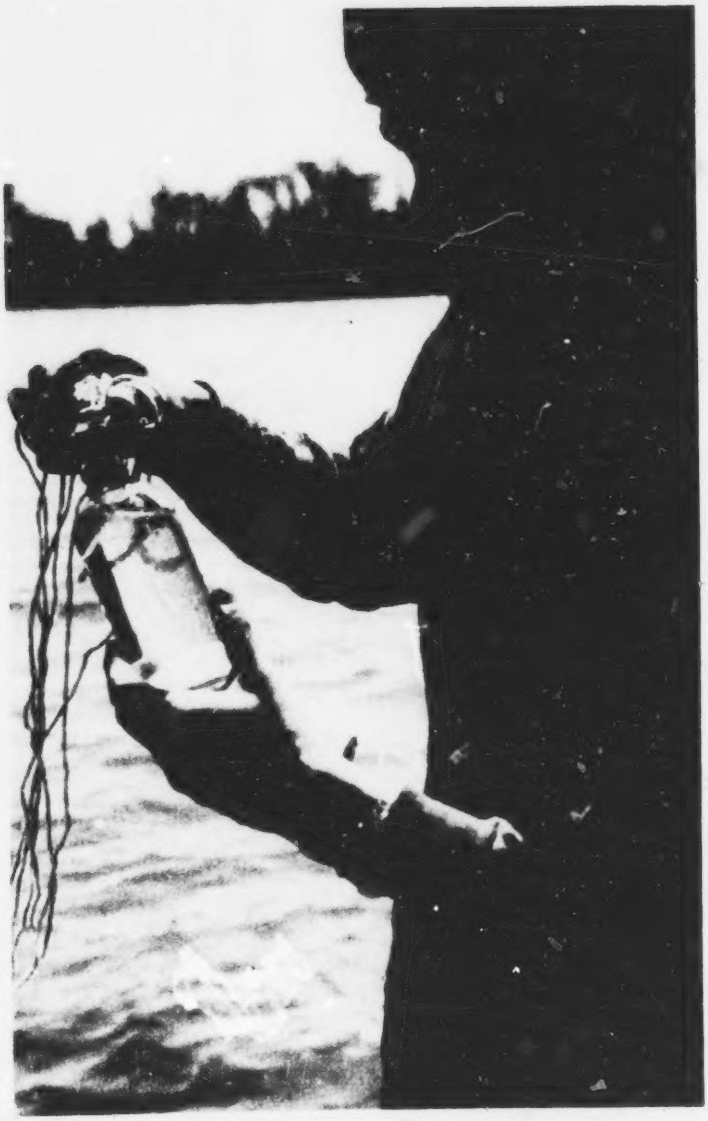


Figure 7. A sterile bottle attached to a light line or string often is resorted to by microbiologists sampling in very shallow waters.

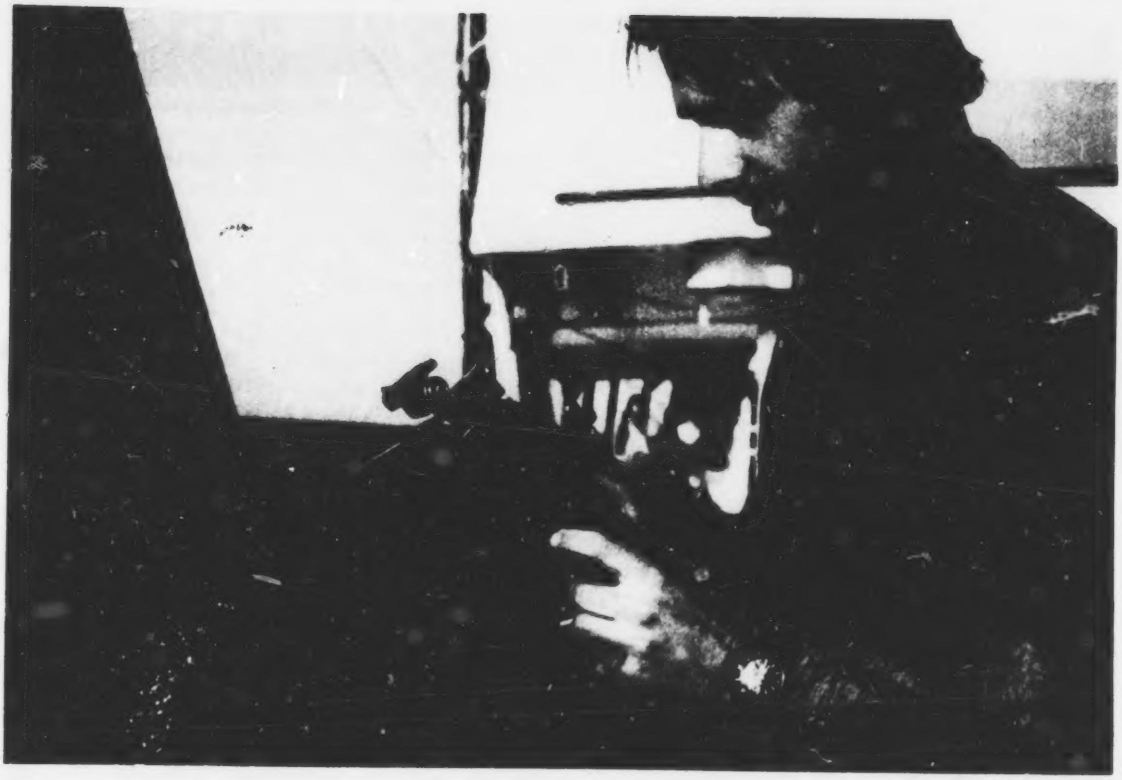


Figure 8. The J-Z sampler devised by ZoBell and his associates (ZoBell, 1946).

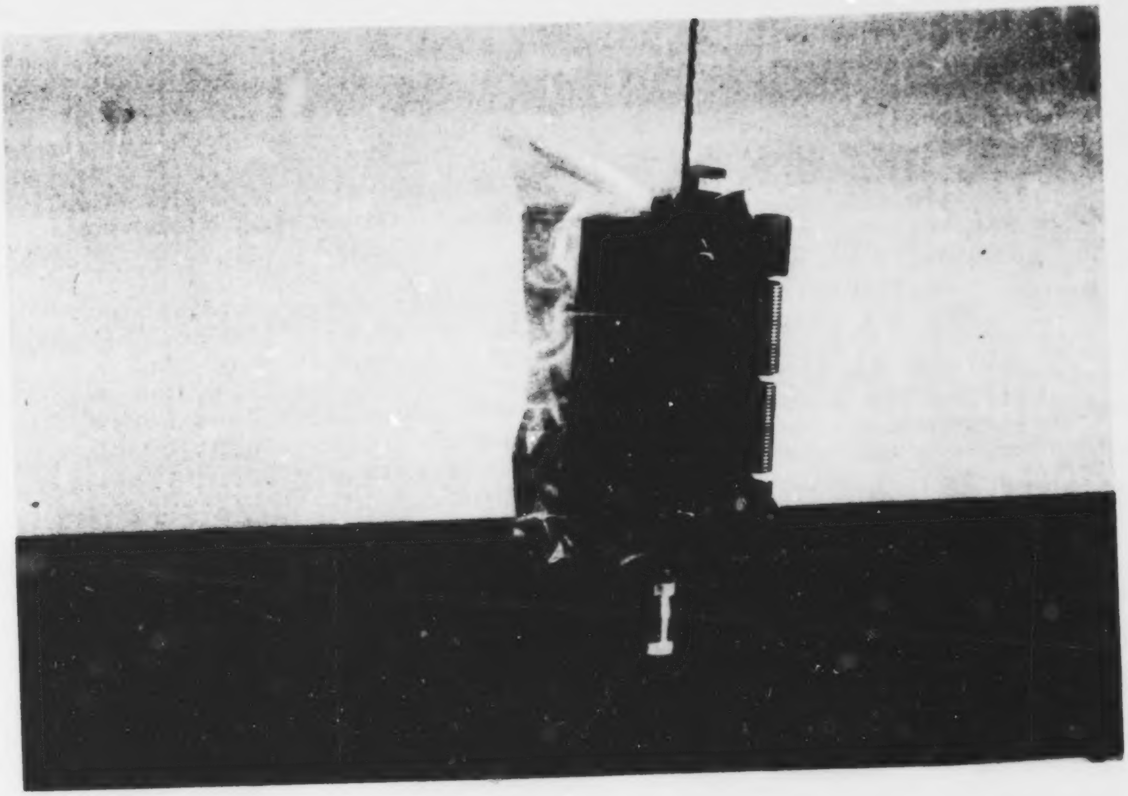


Figure 9. The Niskin sampler. The deep-ocean water sampler most frequently used used by marine microbiologists.

samplers or by hanging samplers one above another, which is not precisely the same as taking multiple, simultaneous samples. Development of such a sampler appears technologically feasible.

There is no sterile sediment sampler available for use in deep waters. Cores are taken by hand in shallow waters or by corers on a hydro-wire. Microorganisms from the surface can be carried down on the outside of the core. After retrieval, however, the core can be frozen in the core liner to prevent further contamination of the inner part. If at all possible, the material should be analyzed immediately (Fig. 10).

A variety of grab samplers have been developed (Figs. 11a, b, c).

A sediment sampler designed by Van Donsel and Geldreich at the EPA Cincinnati laboratory operates in the following manner. The sampler is closed during delivery to the bottom. On contact with the bottom surface, it is opened, taking the sediment sample. A messenger-activated noose then closes the sediment sampling bag. This sampler shows great promise for shallow coastal waters and for estuaries. However, for deeper water, there are problems with opening and closure of this device. Based on our experience, hand-operated samplers are not efficient in waters of depths greater than six feet.

We are presently developing a deep ocean environmental unit which will permit capture and retrieval of deep water samples in a sterile chamber, without alteration of temperature and pressure. Prototype testing is anticipated in early 1974.

Collection of biota for microbiological examination requires dredging or tonging, in the case of molluscs, and trawling for fish. In sampling crabs, some semblance of asepsis can be followed if blood, i.e., haemolymph samples, are required, since sterile hypodermic syringes can be used to withdraw the haemolymph. For neuston, or for floating vegetation, samplers have been devised, but none has been universally accepted. Plankton samples are collected usually by employing nets towed through the water mass under study.

Microbiological water quality monitoring has been considered as preeminently an agar plate and colony counting procedure, following closely the methods used for enumerating coliforms or other indicator organisms. This

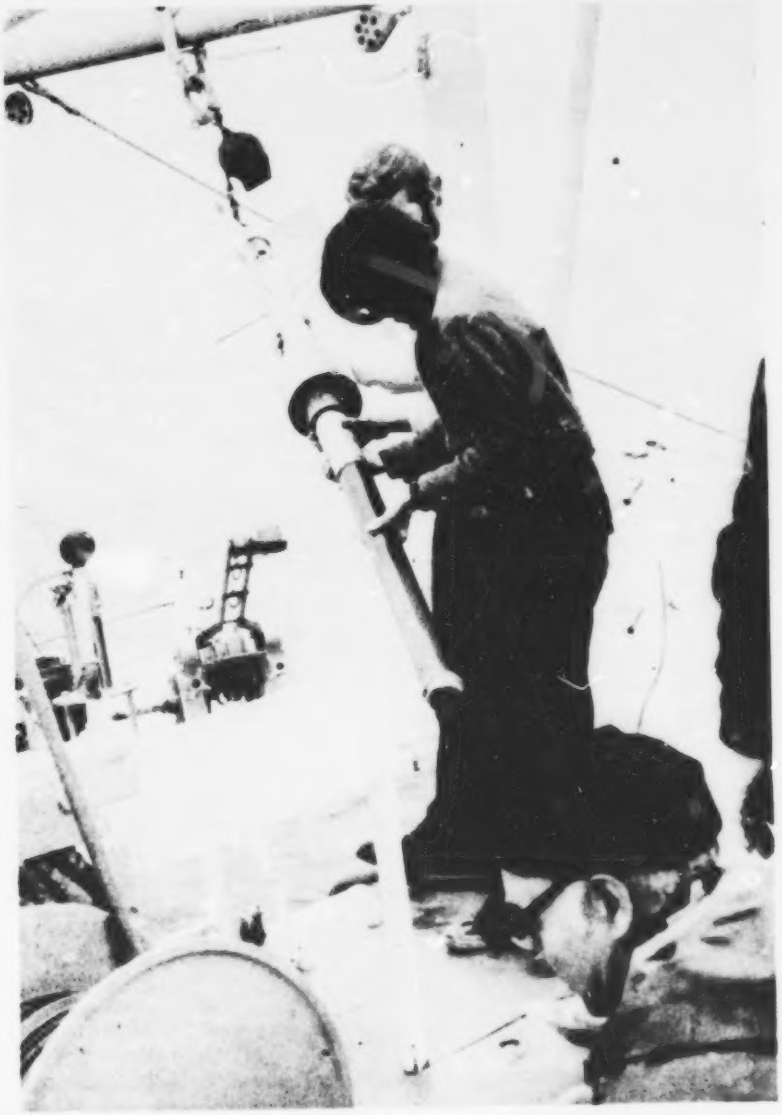
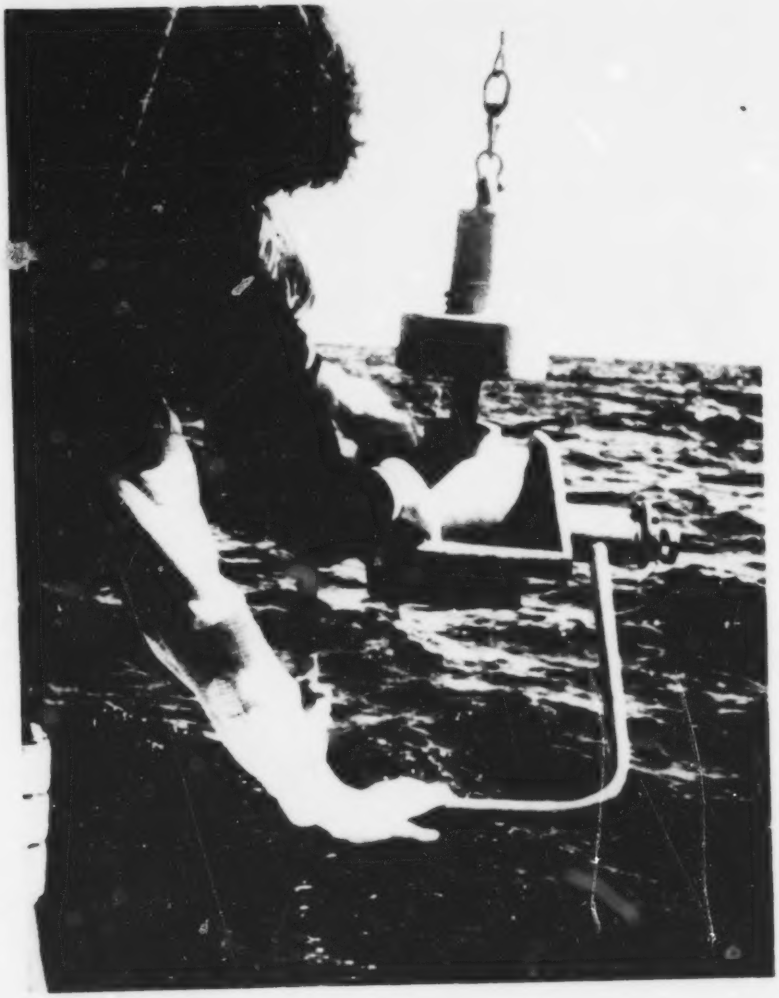


Figure 10. A coring device with a removal, autoclavable core liner. Frequently used to collect sediment samples for microbiological analyses.

Figure 11. a. "Petite Ponar" sampler. (Wildlife Co., Saginaw, Michigan.)



11b. Grab Sampler.

||c. Dredge sampler.

very narrow perspective should be expanded and broadened so that microbiological monitoring can evolve to encompass such techniques as: (1) measuring metabolic activity of mixed microbial populations by ^{14}C uptake of labeled lactose or sucrose, a potentially valuable way to estimate survival and function of enterics in a stream or estuary, and (b) assessing rate of electron transport, using indicators such as tetrazolium salts. With suitable electron acceptor dyes, the activity of NAD or FAD can be measured by visible color change of those dyes (5). To cite an example of the application of such dyes, 2, 3, 5-triphenyltetrazolium chloride (TTC) has been used to distinguish active and inactive bacterial colonies on agar plates in genetic studies. Also, electron transport acceptor dyes or indicators have been used by Hobbie *et al*. (2), to compare results of this technique with measurements of ATP and respirometry of concentrated samples of macroplankton.

A new approach to microbial ecology has been taken in our work whereby the concept of analyzing microbial populations for their species composition has been adopted. In a study of the distribution of *Vibrio parahaemolyticus* in Chesapeake Bay and off the Atlantic Coast, it was observed that *V. parahaemolyticus* is restricted to estuarine regions and undergoes a seasonal cycle in Chesapeake Bay (1, 6). Seasonal fluctuation of bacterial species capable of metabolizing mercury (7) and petroleum (12) is under observation, Figs. 13 and 14. The metabolism of mercury compounds by mercury-resistant bacteria and generic distribution of these bacteria have also been determined (8). The results of these studies have shown that, although total viable counts of sediments and water may not fluctuate significantly throughout the year, the bacterial species comprising these populations demonstrate marked variations which, in general, are correlated with seasonal effects.

In conclusion, sampling technology must be perfected and new methods developed. Environmental microbiological monitoring cannot yet be accomplished with the degree of sophistication required by the complex and complicated demands made of those charged with that task. A second point to be emphasized is that present thinking in "pollution microbiology" ought to be shifted away from rote measurement by plate count of one or two bacterial species of public health significance. The complexities of the estuarine and marine environment are so great that we will always find ourselves caught red-faced because the fecal coliform index or the fecal strep ratio "didn't work." These techniques always "work", but we don't know enough about the seasonality of bacteria and virus fluctuations or

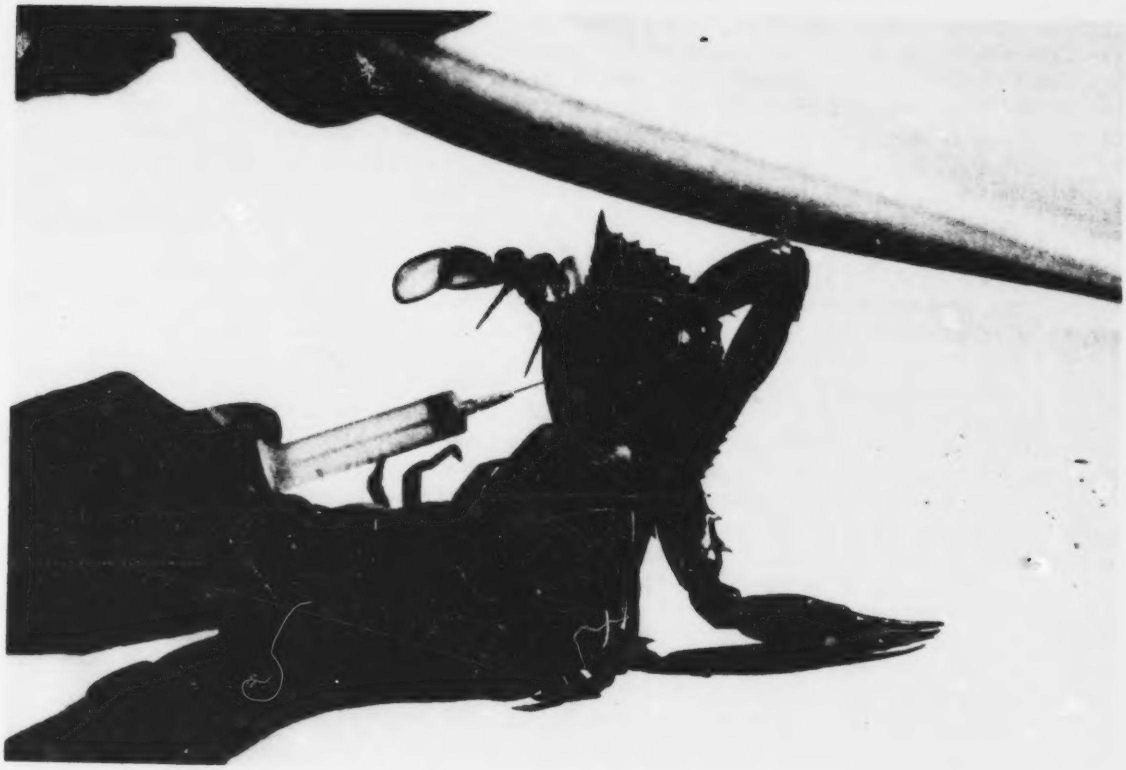


Figure 12. Aseptic removal of haemolymph from a Chesapeake Bay Blue Crab.

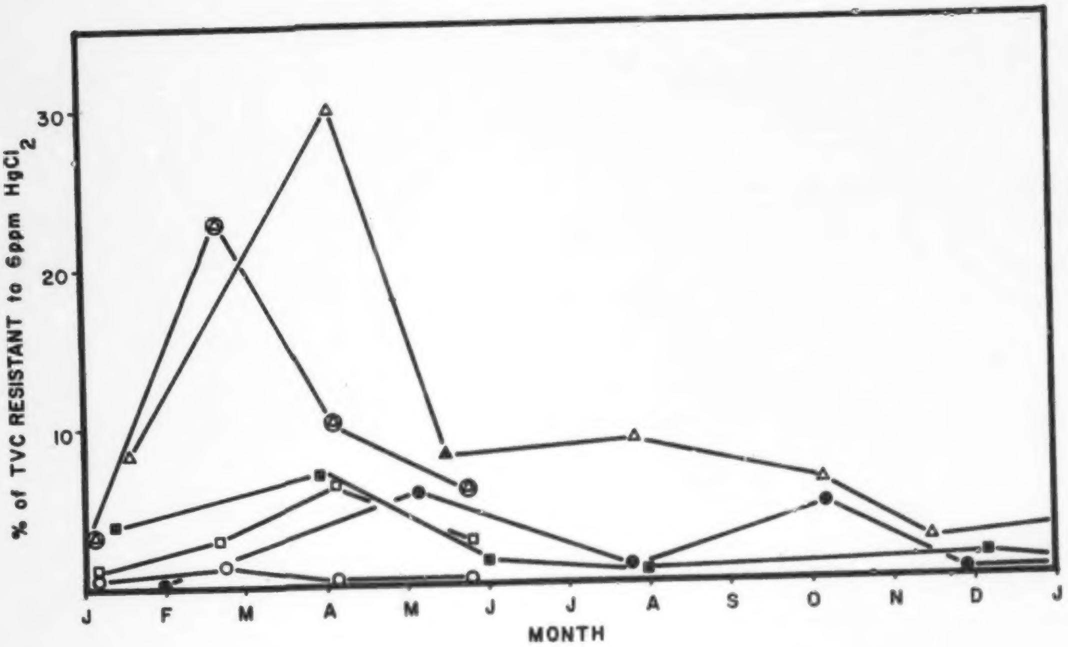


Figure 13. Seasonal variation in percent of HgCl - resistant bacteria in Chesapeake Bay sediment. The percent of total, viable, aerobic, heterotrophic bacterial count (TVC) capable of growth on a solid medium containing 6 ppm HgCl was determined. Sediment samples from Chesapeake Bay Stations B-1, 1972 (▲); B-2, 1972 and 1973 (△,⊖); and EB-1, 1972 (●,○) were plated and incubated at 25 C for 1 week. (Nelson and Colwell, 1973.)

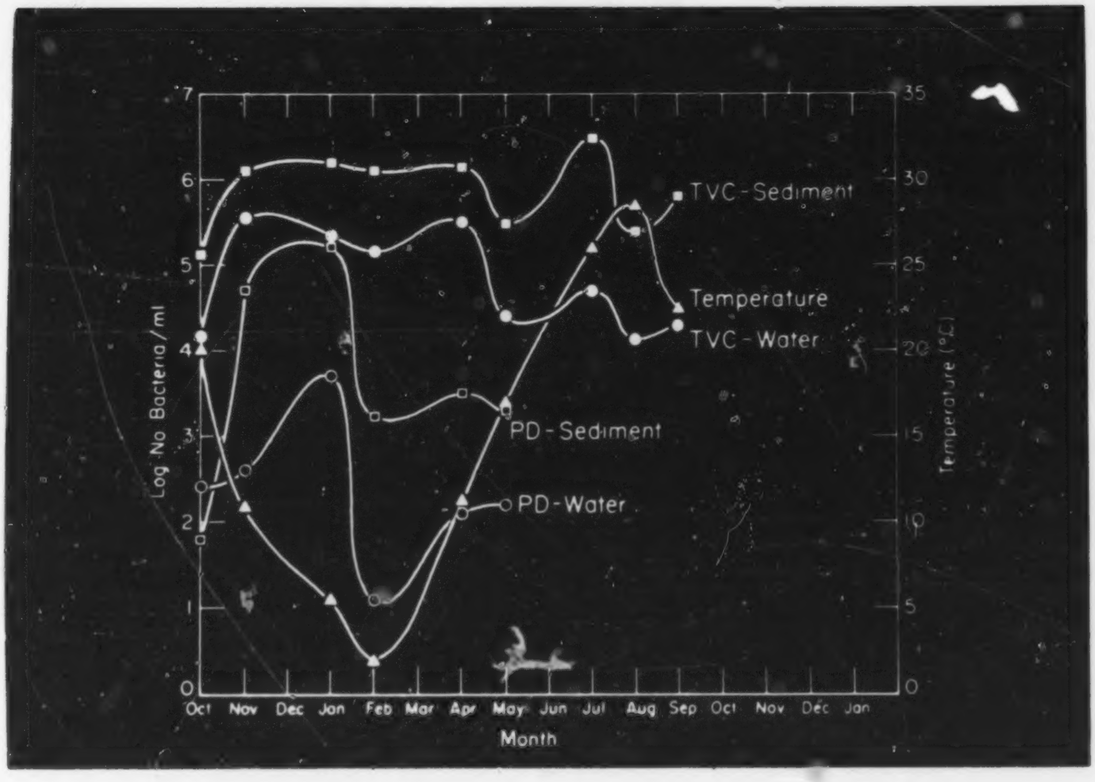


Figure 14. Seasonal variation in petroleum-degrading bacteria in Chesapeake Bay. (Walker and Colwell, 1973.) TVC = total viable count of aerobic, heterotrophic bacteria; PD = petroleum degrading microorganisms.

the microbial ecology of the estuaries and ocean regions to interpret the data obtained, even when these indices are correctly measured. Instead, sharp focus ought to be placed on the bacterial species found in water and sediments and on those distributional variations which follow sedimentation patterns and which are correlated with tides, currents and movements of water masses. Furthermore, the survival and function of microorganisms, both autochthonous and allochthonous, in the sediments and suspended sediments should be studied, for it is in sediments and bottom sludges that "over-wintering" or long-term survival of microorganisms occur and in the suspended sediments that spatial transfer of microorganisms takes place.

Finally, we should re-shape our microbiological approaches so that an understanding of microbial "degraders" and "mineralizers" in the environment is achieved. With such knowledge, these microorganisms can be put to work as well as controlled. *In situ* microbial activities provide the key to microbial ecology, not the static measurement of parameters. The latter comprises the "uncertainty principle" of microbiological sampling, i.e., that which is measured is immediately the past; the present changes as the measurement is made, hence the future, i.e., prediction from the measurement, is uncertain, if not erroneous. Technologically, improvements in the methods for measuring indicator organisms and indices of pollution, such as total coliforms, dissolved oxygen, biological oxygen demand, etc., may eventually result in accuracies to the nth decimal point, but the intrinsic value of these measurements will remain limited, for the simple reason that an index does not tell us enough of what we need to know about a dynamic and ever-shifting environment. Simplistic approaches to environmental problems are inadequate, at best, and potentially disastrous. At the present time, improvement in sampling methods and equipment for obtaining data should be the major, first-order priority.

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MICROBIOLOGICAL METHODS FOR MONITORING MARINE WATERS FOR POSSIBLE HEALTH EFFECTS

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Standardized, quantitative methods for monitorning the marine environment for the range of microorganisms which could cause health effects in man are the exception rather than the rule. This statement is valid if a standardized method is defined as one whose accuracy, precision and sensitivity have been evaluated and found satisfactory with marine environmental samples collected from a number of geographically different areas. This situuation derives in part from limited knowledge as to which health effects indicator or pathogenic microorganisms should be quantified. In turn, this stems from a paucity of epidemiological data relating the incidence of illness to the density of one or more microbial pathogens or indicators in marine waters, the underlying sediments and the fauna and flora therein. For example, most regulatory agencies monitor natural recreational waters for coliform organisms to determine the degree of fecal contamination (26); and there is no question that these waters should be monitored for fecal pollution, since the potential for transmission of enteric disease does exist and operational criteria are necessary. Yet, in Stevenson's (31) epidemiological study on recreational waters, upper respiratory complaints were more common than gastrointestinal disturbances. Another example concerns the requirement for monitoring marine waters for *Vibrio parahemolyticus*, a major causative agent of food poisoning in Japan (15,28) and one which has been isolated from coastal waters and shellfish in the United States (3,14,23). Although *V. parahemolyticus* has been clearly established as the etiological agent of disease outbreaks, there are insufficient data on its relationship to pollution.

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Thus, with reference to microbiological methodology for monitoring the marine environment, the more immediate question concerns the organisms whose enumeration is more likely to provide information on health hazards to recreationists, individuals consuming marine fauna and flora as foods and, possibly seaside populations exposed to aerosols produced by wave action. The sources of these pathogenic microorganisms in the marine environment can be (a) raw and treated fecal wastes from warm-blooded animals, (b) certain industrial effluents, such as those from food processing, wood pulp, textile finishing, and certain chemical plants, and (c) "dumped" materials such as dredged materials, sludge and garbage. In addition, nutrient pollution resulting from industrial and municipal wastes and even thermal pollution could result in the propagation to hazardous levels of aquatic microorganisms capable of producing disease in man. Non-halophilic species, such as *Klebsiella* sp. *Pseudomonas aeruginosa* and *Aeromonas hydrophila*, must be considered along with the halophile, *Vibrio parahemolyticus* since estuarine as well as coastal waters are to be monitored.

SOURCES OF PATHOGENIC MICROORGANISMS

Fecal Wastes

Traditionally the human pathogens of concern in untreated and treated fecal wastes are those excreted from infected individuals or carriers, i.e., enteropathogenic viruses, *Salmonella*, *Shigella*, enteropathogenic *Escherichia coli*, and in some parts of the world the cholera bacillus. However, consideration also should be given to certain "opportunistic" pathogens such as *P. aeruginosa* (19), *A. hydrophila* (17,32), and *Klebsiella* (13) which, although infrequently present in small numbers in human and animal feces, appear to propagate during the course of sewage treatment and can be discharged in large numbers in effluents which are insufficiently treated and chlorinated. *P. aeruginosa* and *A. hydrophila* in densities of about 10^2 and 10^4 per ml respectively, were obtained from unchlorinated effluents at various stages of treatment at each of nine sewage treatment plants examined. Furthermore, the distribution of coliform types appears to change through sewage treatment. Data collected in our laboratory suggest that, concurrent with the decrease in *E. coli* levels through the course of sewage treatment, there is at times an increase in *Enterobacter* and *Klebsiella* densities. Table 1 and Figure 1 show the decrease in *P. aeruginosa* and *A. hydrophila* densities with the increase in the distance

Sampling[1] station	Mean recovery/100 ml	
	P. aeruginosa[2]	Fecal coliforms[2]
N 1	24	1100.
2	24	1500.
3	18	1400.
4	27	490.
5	17	600.
6	7.7	63.
7	4.2	47.

[1] Sampling stations shown in Fig. 1.

[2] Number of samples: 7, collected between February and December.

Table 1. Pseudomonas and fecal coliform densities in Providence River, R.I.

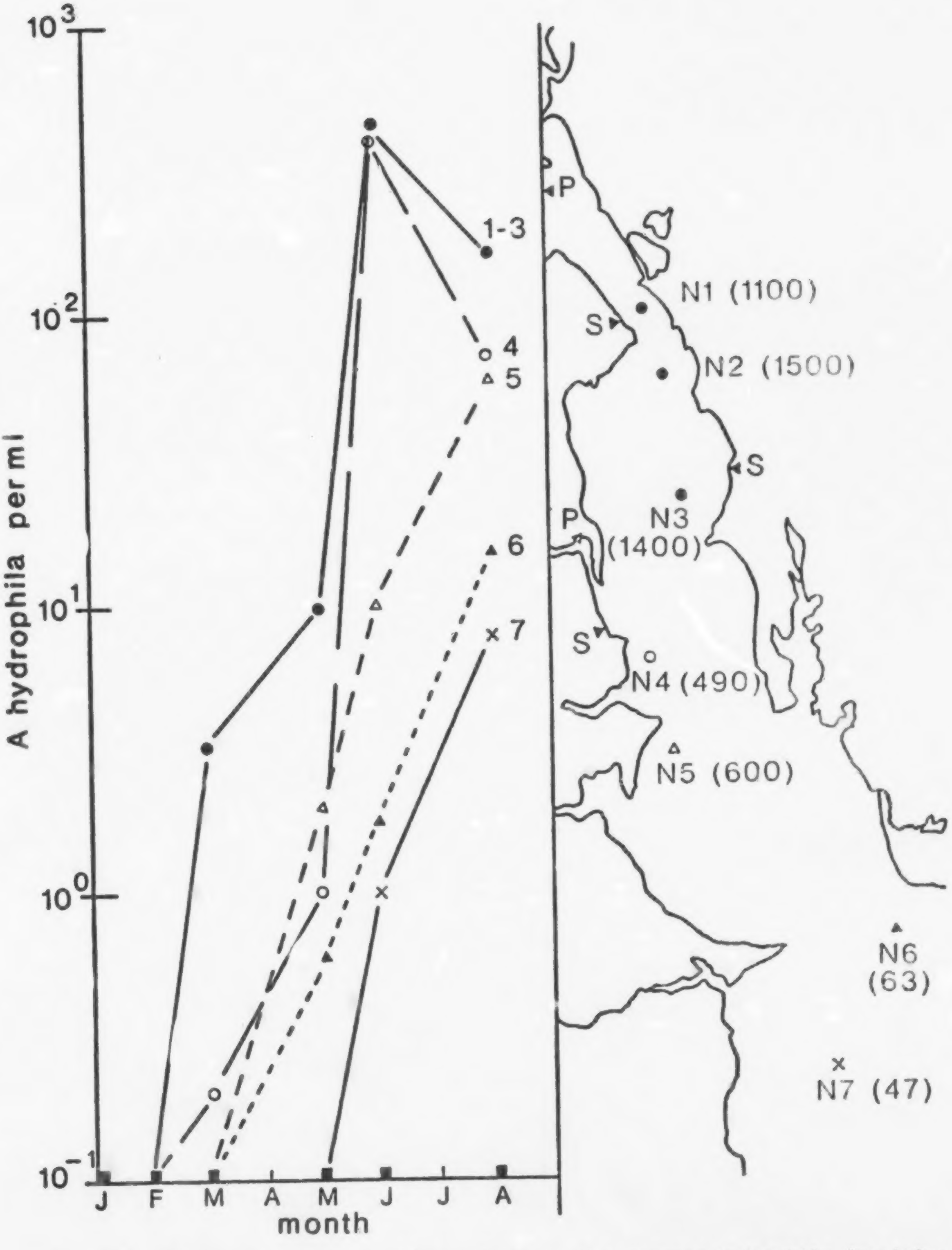


Figure 1. Seasonal and geographic variations of *Aeromonas hydrophila* densities at stations N 1-7 in Providence River and upper Narragansett Bay.

downstream from sanitary pollution sources in the Providence River, Rhode Island.

Industrial Effluents

Klebsiella is both a coliform and an "opportunistic" pathogen which can cause upper respiratory and urinary tract infections in man. High densities of this organism have been recovered from waters receiving the discharges from pulp mills (6). The authors have found large numbers of this organism and *P. aeruginosa*, a major cause of "swimmer's ear", in the effluents from a textile finishing plant and a chemical plant (Table 2.) Since about 50% of the *Klebsiella* isolates we have obtained from industrial effluents would be defined as fecal coliforms, total and even fecal coliform densities in the receiving waters could be misinterpreted as indicating high levels of contamination with the fecal wastes of warm-blooded animals. However, this does not preclude the need to measure *Klebsiella* densities in waters receiving such effluents, since this organism is a human pathogen. Furthermore, it has been observed that the frequency of mouse pathogenic *Klebsiella* strains isolated from textile finishing plant effluents was no less than that from clinical sources (10).

Aquatic Mcroorganisms

At least four aquatic microorganisms, *P. aeruginosa*, *A. hydrophila*, *K. pneumoniae*, and *V. parahemolyticus* are capable of causing disease in man and multiplying in the aquatic environment. All four of these organisms can be found in significant numbers in estuarine waters, depending, of course, upon seasonal and other factors. Additional data are needed on the relationship of the levels of these organisms to the proximity of nutrient pollution sources.

Significance of Sources

The sources of human pathogens present in the estuarine environment are summarized in Table 3. With the exception of the enteropathogenic viruses, *Salmonella* and *Shigella* species, the remaining pathogens all have extra-fecal sources. If epidemiological data do reveal significant health hazards associated with their presence at given densities, these hazards may not be "indicated" by coliforms alone. Even with reference to those microbial pathogens which have no extra-fecal source, several investigators, most recently Dutka (12), have questioned the validity of recreational water standards based on coliforms and even fecal coliforms. Dutka (11) cited several instances in

Organisms	Recovery/100 ml in effluents from Textile Finishing Plants	Chemical Plants
Total Coliforms	5.3×10^7	7.0×10^5
E. coli	$< 10^5$	1.0×10^5
Klebsiella	2.7×10^7	6.0×10^5
Enterobacter	2.6×10^7	$< 10^5$
Citrobacter	$< 10^5$	$< 10^5$
P. aeruginosa	1.3×10^5	5.8×10^6

Table 2. Distribution of coliform types in industrial effluents

	Source of the organism			
Pathogen	Feces	Indust.	Run-off	Aquatic
Enteroviruses	+	-	-	-
Salmonella	+	-	-	-
Shigella	+	-	-	-
A. hydrophila	+	-		+
P. aeruginosa	+	+	+	+
V. parahemolyticus	-	-	-	+
C. botulinum	-	-	+	+

Feces and sewage wastes (30, 5, 24).

Certain types of industrial effluents, i.e. pulp mills (4).

Run-off from "uncontaminated soils" (24).

Multiplication in the aquatic environment reported (5, 24, 21).

Table 3. Sources of some pathogenic bacteria found in the marine environment

which there was no consistent relationship of coliform or fecal coliform levels to Salmonella densities or in which salmonellae were isolated from waters with very low coliform densities.

The question of the consistency of coliform-enteropathogen relationships is the problem of extra-fecal sources of the more commonly used indicators of fecal pollution. These are shown in Table 4. The implication from this table affirms what several workers in the field have pointed out, i.e., that E. coli is the most specific indicator of pollution from the fecal wastes of warm-blooded animals (12). However, in monitoring marine waters for the range of pollution sources, it would be more illuminating to obtain data on the distributions of the "component genera" of the coliform population. A procedure for obtaining such data will be described later in this report.

STATUS OF MICROBIAL METHODOLOGY

The status of quantitative, microbiological methods evaluated for use in monitoring the marine environment is presented in Table 5. Available methods were categorized either as a Standard Method (SM), i.e. one which appears in Standard Methods for the Examination of Water and Wastewater (2) or in the Bacteriological Analytical Manual published by the Division of Microbiology, Food and Drug Administration (1), as a method which has been designated as tentative (TSM) in Standard Methods for the Examination of Water and Wastewater, or as an experimental method which has received some degree of evaluation with samples collected from the marine environment. The term "None" is shown for those instances in which there is no method meeting the above criteria. Standardized enumerative methods for routine monitoring may not be required for all the organisms listed in Table 5. Rather, these organisms should be considered in determining which microbiological measurements should be made to obtain the data needed to derive criteria and standards. The state of the art with regard to methods for monitoring the marine environment is overstated in Table 5. Thus, in several instances where there are "Standard Methods" for quantitating microbial pathogens or indicators, such as P. aeruginosa, total coliforms, fecal coliforms, and fecal streptococci, the methods have not been sufficiently evaluated with samples from marine waters. For example, the method for P. aeruginosa, as given in the current edition of "Standard Methods", has been evaluated and found unsatisfactory for use with marine waters. It is being replaced with a membrane filter (MF) method (24).

Bacterial Indicator	Source of organism		
	Indust.	Run-off	Aquatic
Coliforms	+	+	+
E.coli	-	-	+ or -
Klebsiella	+	+	(+)
Enterobacter	+	+	(+)
Citrobacter	-	+	(+)
Fecal coliforms	+	+	(+)
Fecal streptococci	-	+	

Indust. - certain types of industrial effluents (4).

Run-off from "uncontaminated soils" (9, 16, 25).

Multiplication in the aquatic environment reported (30, 7, 4, 20).

Portion of coliform population which may multiply.

Table 4. Sources other than feces for bacteria used as indicators of fecal pollution

Organism	Water	Collection from Sediment	Fauna
Enteroviruses	Exp.	None	None
Salmonella	None[1]	None	None
Shigella	None	None	None
P. aeruginosa	TSM[1]	None	None
A. hydrophila	None[1]	None	None
Coliforms	SM[1]	SM[2]	SM[2]
E. coli	None[1]	None	None
Klebsiella sp.	None[1]	None	None
Enterobacter	None[1]	None	None
Citrobacter	None[1]	None	None
Fecal coliforms	SM[1]	SM[2]	SM[2]
Fecal streptococci	SM[1]	SM[2]	SM[2]
V. parahemolyticus	Exp.[1]	SM[2]	SM[2]
C. botulinum		SM[2]	SM[2]
C. perfringens		SM[2]	SM[2]

SM "Standard Methods for the Examination of Water and Wastewater" (2), FDA method (1).

TSM Tentative "Standard Method".

Exp. Evaluated experimental method.

None No method evaluated with marine samples.

[1] Experimental procedure developed or under development for marine recreational waters.

[2] FDA method assumes isolation methods can be used in MPN method for quantification.

Table 5. Quantitative, microbiological methods evaluated for use in the monitoring of marine environment

Unpublished data from several laboratories concerned with the examination of marine water samples have indicated that the mEndo and mFC procedures for the enumeration of total and fecal coliforms, respectively, underestimate these populations. Samples of the data collected in our laboratory which support this contention are shown in Table 6, which compares the recovery of total coliforms by the mC and mEndo procedures and Table 7 which compares mC to the mEndo and "standard" MPN methods for the recovery of total coliforms. Tables 8 and 9 provide the same comparisons for fecal coliform recoveries obtained by the mC, mFC and MPN procedures. With 47 estuarine water samples collected from Raritan Bay, fecal coliform recoveries by the MPN method were about twice those by the mFC procedure, on the average.

The KF method, which some investigators recommend as providing the best estimate of fecal streptococci in fresh water, was found to markedly underestimate the densities of this group of bacteria in marine waters in the northeast United States. This can be seem from Table 10 which compares the densities of fecal streptococci by the KF method to those by the mSD procedure developed in our laboratory (25).

There remains some question whether the microbial methodology for use with sediment and faunal samples as indicated in Table 5 will, in fact, provide quantitative estimates of the accuracy and precision required. Most of these methods are or can be used as multiple tube procedures which yield most probable number (MPN) estimates, with the attendant problems of precision or reproducibility. Furthermore, with faunal, sediment and even turbid water samples, it is not certain whether single cells or particles composed of one or more viable organisms are being counted.

ON-GOING METHODS RESEARCH

Studies towards the development of microbiological methods specifically applicable to monitoring the marine environment for health effects microorganisms are on-going at a few laboratories. Those organisms for which methods are being developed at our own laboratory are indicated in Table 5 with the superscript "1". The major thrust of our effort has been to obtain accurate and precise methods for use in epidemiological-microbiological trials being conducted to establish criteria for recreational water. It is beyond the scope of this report to describe all the methods which have been developed or are undergoing development. However, some examples are given to illustrate

Sample source	Recovery per 100 ml by mC	mEndo
Providence River	2000	1100
	5300	3200
	1500	550
	120,000	90,000
Boston Harbor	290	140
	100	70
	14	8

Table 6. Comparison of confirmed coliform recoveries from marine waters by mC and mEndo procedures

Relationship	Comparison of recoveries[1] by mC to mEndo	MPN
No. of samples	33	20
mC > by 5%	25	14
mC < by 5%	7	6
< 5% diff.	1	0

[1] Confirmed by picking colonies to lactose broth.

Table 7. Confirmed coliform recoveries from New York Bay by the mC procedure as compared to those by the mEndo and MPN methods

Sample source	Recovery per 100 ml by fecal mC	mFC
Providence River	2200 7700	280 1000
Boston Harbor	50	6
New York	2100 6000	1100 1000

Table 8. Comparison of confirmed fecal coliform recoveries from marine waters by fecal mC and mFC procedures

Relationship	Comparison of recoveries by mC to mFC	EC MPN
No. of samples	16	16
fecal mC > by 5%	12	8
fecal mC < by 5%	3	6
< 5% diff.	1	2

Table 9. Confirmed fecal coliform recoveries from New York Bay by the fecal mC procedure as compared to those by the mFC and EC MPN methods

Conf. Rec./100 ml		mSD/KF	Conf. Rec./100 ml		mSD/KF
mSD[1]	KF		mSD	KF	
404	8	50.	46	5	9.2
243	6	40	7	1	7.
150	6	25.	32	5	6.4
47	2	24.	38	8	4.8
70	4	18.	14	4	3.5
230	17	14	34	13	2.6
153	12	13	121	50	2.4
46	4	12	25	43	.58
310	26	12	13	61	.21
10	1	11.			

Median mSD/KF ratio 11.

[1] In excess of 95% of the isolates identified as S. fecalis, S. faecium, S. durans or S. zymogenes.

Table 10. Comparison of "fecal streptococci" densities in New York City beaches by the KF and mSD procedures

Organism	Recovery per 100 ml at Coney Island (19th St.)	 Rockaways (67th St.)
Total coliforms	2490	58
Fecal coliforms	453	28
E. coli	403	24
Klebsiella	356	12
Enterobacter	1678	15
Citrobacter	67	2
P. aeruginosa	45	7.6

Table 11. Distribution of coliform types at New York City beaches

Organisms	Recovery/100 ml in water at Site 1	 Site 2	 Site 3
Total coliforms	2.1×10^4	3.1×10^6	5.6×10^4
Fecal coliforms	-	3.1×10^6	2.8×10^4
E. coli	2.1×10^4	$< 10^5$	$< 10^3$
Klebsiella	$< 10^3$	3.1×10^6	2.8×10^4
Enterobacter	$< 10^3$	$< 10^5$	2.8×10^4
Citrobacter	$< 10^3$	$< 10^5$	$< 10^3$
P. aeruginosa	35	41	8

Table 12. Distribution of coliform types at fresh water sites

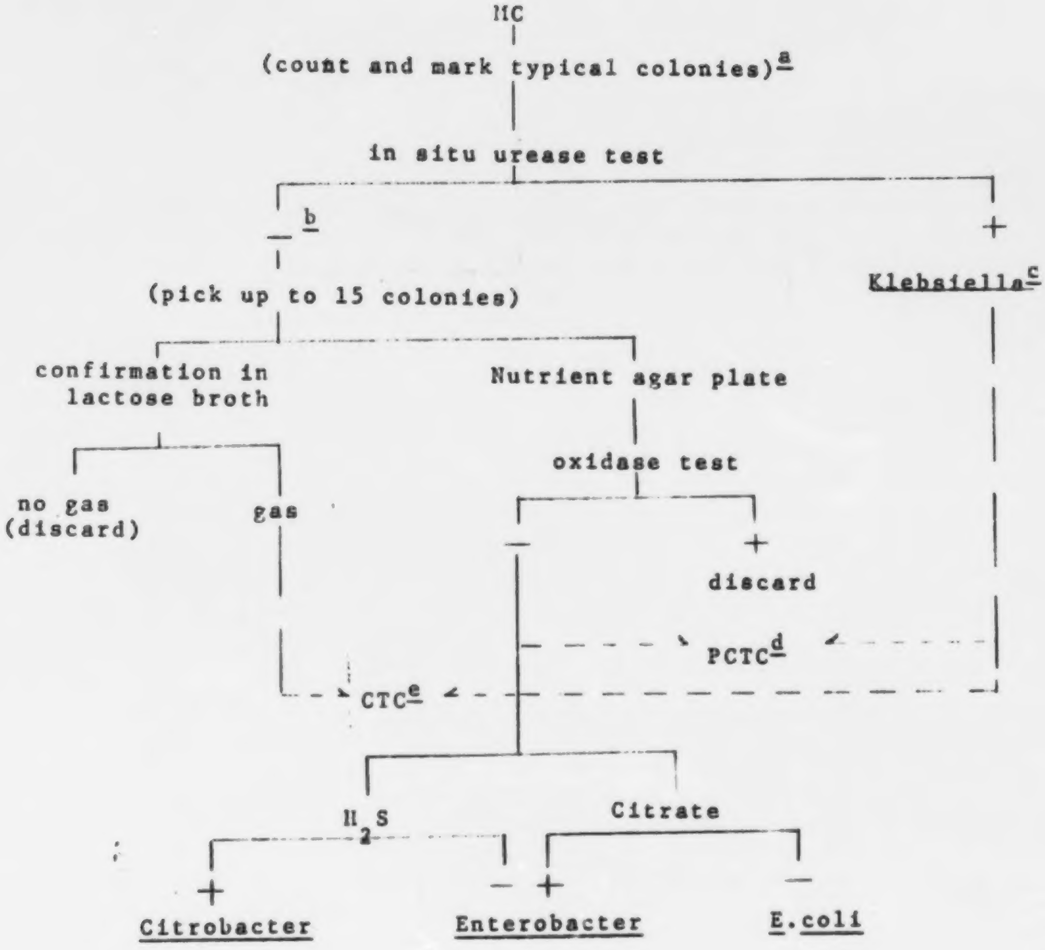
the direction of the research and the types of information which can be obtained thereby.

Coliforms

A membrane filter procedure for the quantification of coliforms in marine waters has been developed (10). In the development of the method (mC) certain prerequisites were considered: (a) the method should permit the examination of large quantities of water and provide more precise estimates than can be obtained with MPN procedures; (b) it should allow the option of measuring total coliforms, fecal coliforms and component genera of the coliform population; (c) an oxidase test should be included to exclude Aeromonas hydrophila, since some strains of this organism are lactose positive and aerogenic; and (d) except when confirmation is required, "picking" of colonies for biochemical or serological identification should be minimized. The achievement of the last prerequisite has been furthered by use of in situ substrate tests in which the membrane filter, following incubation on a selective-differential medium, is sequentially transferred to one or more substrates containing appropriate indicator systems. It can be seen that in the mC procedure, as shown in Figure 2, colonies are picked for identification. However, encouraging results have been obtained by following the in situ urease test for Klebsiella with an in situ oxidase test to eliminate A. hydrophila and then an in situ indole test, such as suggested by Delaney (8) for differentiating Citrobacter and Enterobacter from E. coli. The type of information which can be obtained from the mC procedure is shown in Table 11. The E. coli to Enterobacter ratios indicate that the sources of pollution reaching the two beaches are different. In this instance the fecal coliform-total coliform ratios would have provided the same insights. Such would not be the case in waters receiving industrial effluents containing large numbers of Klebsiella. The occurence of water samples in which Enterobacter, Klebsiella, or E. coli constitute the major component of the coliform population is shown in Table 12.

Fecal Streptococci

A flow diagram for the conduct of the mSD method for fecal streptococci is shown in Figure 3. In this procedure, incubation at 41 C on a medium highly selective for Group D, streptocci (to date, neither Streptoccus salivarius, S. mitis, S. equinus, and S. bovis has been recovered) is followed by an in situ esculin test. Fluorescent antibody staining can be used to confirm and identify the isolates.


MC
(count and mark typical colonies)[a]
in situ urease test
— [b]
+
Klebsiella[c]
(pick up to 15 colonies)
confirmation in lactose broth
Nutrient agar plate
no gas (discard)
gas
oxidase test
—
+
discard
PCTC[d]
CTC[e]
H_2S
Citrate
+
Citrobacter
— +
Enterobacter
—
E.coli

[a] Presumptive totalcoliforms

[b] Urease negative coliforms (UNC)

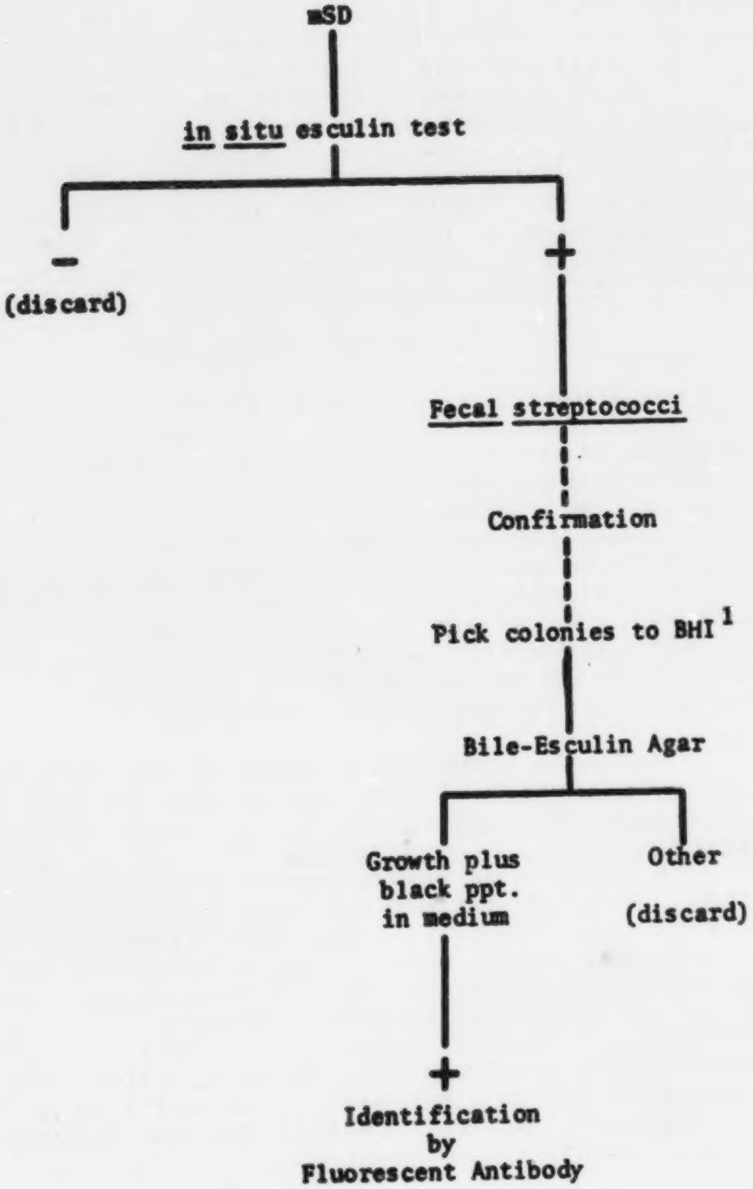
[c] Typical, urease positive colonies, rarely do not confirm

[d] Partially Confirmed Total Coliforms = UNC $\frac{\text{Oxid. neg.}}{\text{Total picked}}$ + Klebsiella

[e] Confirmed Total Coliforms = UNC $\frac{\text{oxid neg, lact. pos.}}{\text{Total picked}}$ + Klebsiella

– – – for calculating coliform densities

Figure 2. Flow diagram for total coliforms and distribution of coliform types. Fecal coliform estimate can be obtained by performance of EC test from lactose positive confirmatory tubes.


mSD
in situ esculin test
−
(discard)
+
Fecal streptococci
Confirmation
Pick colonies to BHI[1]
Bile-Esculin Agar
Growth plus black ppt. in medium
Other
(discard)
+
Identification by Fluorescent Antibody

[1] BHI - Brain Heart Infusion broth.

Figure 3. Flow diagram of mSD procedure for fecal streptococci.

Aeronomas hydrophila

The objective of eliminating the picking of colonies for identification has not been achieved with this procedure. None of many compounds tested were found to completely inhibit coliforms and other organisms present in marine waters while allowing quantitative recovery of *A. hydrophila*. Incubation of the filters at 37 C, an *in situ* oxidase test, and transfer of typical colonies to double sugar iron agar (containing mannitol as the "major" sugar, inositol as the "minor" sugar and no NaCl) permits the differentiation of *Aeromonas hydrophila* from coliforms, non-fermentative pseudomonads and achromobacters, salt requiring vibrios, *Aeromonas salmonicida* and *A. shigelloides*.

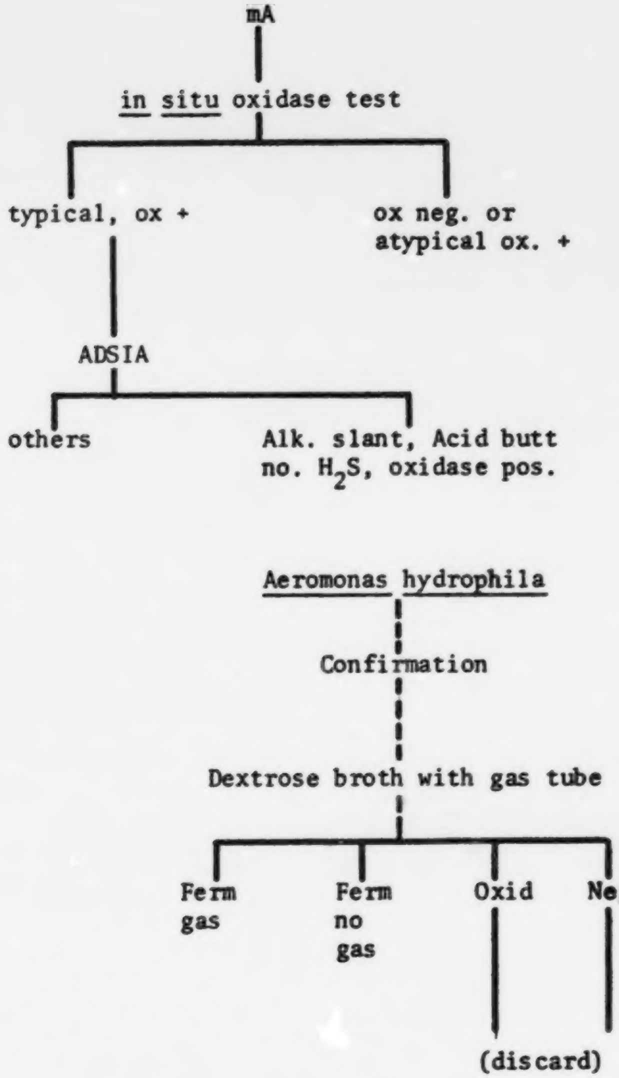
NEEDED METHODOLOGY

As evaluated, a reasonably facile and minimally destructive method is needed for dispersing multicell particles into single cell units and separating the microorganisms from sediment particles to which they adhere. The resulting microbial suspensions then could be assayed by conventional plating or MF procedures. In addition, an assay for the spores of *Clostridium perfringens* could provide a sensitive means of monitoring the dispersal and persistence of sewage sludge and, possibly, dredge spoils "dumped" into marine waters. Methods are available for the enumeration of marine sediments, especially if the aforementioned procedure is developed.

It has been shown by Miyamoto *et al*. (27) with *Vibrio parahemolyticus*, by Kauter and associates (22) with *Clostridium botulinum*, and in our own laboratory with *Klebsiella* (23), *A. hydrophila* (5), and *V. parahemolyticus* that the numbers of environmental isolates which are "pathogenic", as defined by mouse pathogenicity or toxigenicity, generally constitute minor portions of the populations. Since some of these organisms are opportunists, it may be that an "unstressed" mouse is not the animal of choice to model pathogenicity for "susceptible" humans. More likely, only a portion of the environmental isolates are, in fact, pathogenic; and the enumerative methods may have to be modified so that they are both selective and differential for the "pathogenic" biotypes within these species.

DISCUSSION AND CONCLUSIONS

The state of the art concerning methodology for the quantification of health effects indicators in the marine


mA
in situ oxidase test
typical, ox +
ox neg. or atypical ox. +
ADSIA
others
Alk. slant, Acid butt no. H2S, oxidase pos.
Aeromonas hydrophila
Confirmation
Dextrose broth with gas tube
Ferm gas
Ferm no gas
Oxid
Neg
(discard)

Ferm - acid inside and outside Durham tube.

Oxid - acid outside Durham tube.

Neg - alkaline throughout.

ADSIA - trypticase, 1.5%; yeast extract, 0.3%; polypeptone, 0.5%; inositol, 1.0%; mannitol, 0.15%; ferric sulfate, 0.02%; sodium thiosulfate, 0.03%; phenol red, 0.003%; agar, 1.2%. Sterilized and prepared, dispensed, inoculated as described for kligler iron agar.

Figure 4. Flow diagram of mA procedure for aermonas.

environment must be upgraded. The situation stems from several factors:

1. a paucity of epidemiological data relating health hazards to individuals associating with the marine environment to some indicator or indicators of the water quality.

2. insufficient evaluation of existing methodology when applied specifically to the enumeration of the organism in the marine environment.

3. the willingness, in the past, to rely too heavily on coliforms and even fecal coliforms as the sole indicators of water quality.

Efforts are being made to correct this situation. A long-range research program to develop criteria for marine recreational waters is in progress. Central to this effort are epidemiological-microbiological studies to relate the incidence of illness among swimmers to some health effects indicator(s) of water quality at the beaches. A series of trials to evaluate the epidemiological and available microbiological methodology have been completed. In addition, these trials will produce a limited amount of data - the first such data to become available since Stevenson's (31) studies - comparing the rates of illness by type and severity among swimmers and non-swimming controls. The study was conducted at two beaches that were markedly different in their pollution levels according to existing criteria. Methods such as those described herein were used to enumerate a number of possible microbial indicators of water quality. However, until a sufficient quantity of such epidemiological-microbiological data becomes available, we must rely on existing criteria, standards and monitoring procedures as being the best available.

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A SURVEY OF METHODS FOR MONITORING ECOLOGICALLY IMPORTANT MICROORGANISMS IN THE MARINE ENVIRONMENT

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Monitoring implies keeping track of something that warns or instructs relative to a specific purpose: i.e., how much of what is where. The time element, however, compounds the problem. Continuous monitoring may provide a rate of change measurement but does it say what is happening? And how often and for how long is monitoring carried out? Some additional definitive insight is offered by a task group of the National Academy of Sciences (1971). In speaking to the question of the effects of pollutants on marine organisms, and depending on the level of interest (i.e., from cellular level to community dynamics), a span of minutes to decades may be appropriate. Thus between 10 minutes and 10 years, a 5×10^5 span in differences in various monitoring procedures may exist.

A more significant concern may be that of what constitutes an "ecologically important" microorganism. If those organisms which have been monitored on the basis of ability to detect, convenience, or tradition are truly ecologically important, surely others have been treated as ecologically unimportant. More likely there are levels of importance. Many studies have shown the distribution of a wide variety of microorganisms in marine and estuarine waters. The question is, what are they doing?

Consider a bacterium in an estuary which supports a shellfish population; perhaps a species of Flavobacterium, Achromobacter, or some other common nonindicator or nonpathogenic genus. With no other information, we probably would not seek to monitor the presence of these bacteria and could consider them ecologically unimportant. However, shellfish do accumulate bacteria (e.g., Cabelli and

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Hefferman, 1970) and thus assume an ecological significance in terms of mollusc nutrition. Further, newer information indicates that some strains of "common" bacteria can transform environmentally critical compounds such as PCBs (Ahmed and Focht, 1973a,b) and chlorinated hydrocarbons (Leshniowsky *et al*., 1970; Pfaender and Alexander, 1972; Sethunathan and Yoshida, 1973). In this context, certain "unimportant" genera now do assume an ecological importance. Going one more step, the observation of biomagnification in *Aerobacter aerogenes* and *Bacillus subtilis* is significant (Johnson and Kennedy, 1973). Here are two very common, "low-profile" bacteria in which cellular residue magnification factors were up to 4,300 fold compared with water. For subsequent food chain events we now have very ecologically important microorganisms. On microbiological grounds, the shellfish may be safe but unless chemical standards are utilized, high pesticide levels will be present in marketable products as a result of microbial activity.

To be sure, much of these data are derived from *in vitro* studies and therein lies another problem. What about *in situ* activities-what is the level of correlation between the two or, as above, they are there-what are they doing?

Considerable attention has been directed to potential ecological catastrophy, e.g., Torrey Canyon, Santa Barabara oil spill, massive offshore dumping and the like. There is no doubt that these are major events but should we not be concerned also, on a day to day basis, with the endemic, chronic, almost "accepted" addition of materials to the aquatic environment? Included here are local sources of effluents, marinas (Mack and D'Itri, 1973), fuel depots, near-shore dumping (Biggs, 1968), small manufacturing plants and so forth. For example, some monitoring of particular areas has shown the presence of nontraditional microorganisms of possible use as indicators of hydrocarbons (Turner and Ahearn, 1970) and human sewage (Cook, 1970). From studies of these sites seeking specific types of organisms, we may be able to know what to look for in catastrophies. One point is that, while we cannot predict a major oil spill, we do know the siting of sewage outfalls, power plants, refineries, chemical plants, etc. in advance of construction and can monitor these if we know what to look for. Moreover, it can be done in an atomosphere of logic over a long "before" period of time rather than in a panic following an "after" ecological tragedy such as a major oil spill. Baseline studies, for as long as possible, cannot be overemphasized.

Any consideration of methods for assessing anything must presume that the techniques being used (a) do detect the organisms sought and (b) that the organisms, once detected, do indicate what we assume they do. There are, of course, "standard methods" for the examination of water and wastewater (APHA et al., 1971; EPA, 1971) and dairy products (Walter, 1967) and "recommended methods" for the consideration of foods (Sharf, 1966) and seawater and shellfish (APHA, 1970). These are desirable in the sense that at least data from various samples and areas are comparable.

The choice of microbiological methods to be used for a particular circumstance will have to be based on experience. Obviously no research group can monitor all microorganisms at all times; options will have to be exercised. The state-of-the-art at present is such that each study, as an individual case, should consist of certain basic procedures and yet allow for experimentation with new methods. We know, for example, that marine bacteria are essentially psychrophilic and quite susceptible to relatively small temperature change (Morita, 1966). In an estuary, these populations will be mixed with eurythermal types from freshwater or soil sources. One should also consider the salinity effect; to what extent are marine bacteria tolerant of inorganic dilution and how do river or terrestrial bacteria survive a seawater gradient? To examine adequately the ecological role of any of these organisms will require a variety of methods and cultural manipulations and demands both field and laboratory integration. To be sure, the standard laboratory plating techniques can be be employed with a battery of selective and differential media and temperatures of incubation. But this is rather of an all or nothing result. There are, however, some interesting variations on the common plate count theme. Agar "dip-slides" (Mara, 1972) can be used to approximate total and coliform counts for routine monitoring. A double medium technique can be used to study stressed cell injury. One medium allows growth of uninjured cells only while a second shows growth of both uninjured and injured cells. Using lack of colony formation on the former as an index of unrepaired injury, recovery can be detected on the latter medium. This technique has been used for studying lethal and inhibitory effects of sodium chloride on thermally stressed bacteria (Erwin and Haight, 1973). Surely, wider application to environmental problems can be imagined.

A more sophisticated supplement to plate counts is offered by the use of continuous culture or chemostat techniques whereby substrate level, temperature, salinity, pH, Eh, etc. can be managed on a sliding scale to attempt to simulate field observations on special environmental additives in question. Within this laboratory scheme, microscopy, sophisticated biochemical analyses, and other techniques can be superimposed. Emphasis must be placed on the consideration of each situation as unique. The exact choices of monitoring methods and alterations therein are dependent on geographic location of the site, uses (real or potential) of the water, existing hydrographic conditions, and degree of anticipated environmental stress. Because of this, our degree of acceptance of standard methods for the examination of ecologically important microorganisms in the marine environment is questionable.

In any monitoring situation, we look normally at a particular core of organisms, both micro and macro. Among the former are the bacteria and phytoplankton. Recent studies on phytoplankton indicate that, in some areas, the dominant organisms are the very small one (Malone, 1971; NOAA, 1972; Reynolds, 1973) and easily missed by traditional techniques. Considering the indicator potential of microalgae in general in ecological assessments of thermal (e.g., Patrick, 1969; Lanza and Cairns, 1972) and chemical (Carpenter et al., 1972; Brook and Baker, 1971; Hamilton et al., 1970) additions, we may well be overlooking the major portion of the plankton biomass.

In our own laboratory, we have studied the effects of thermal addition (Buck and Rankin, 1972; Foerster et al., 1974), treated sewage effluent (Pilcher and Buck, 1973), and dredge spoils (Bireley, thesis in preparation) on, respectively, a river, an estuary, and Long Island Sound. Using the "usual" parameters of total bacterial counts and numbers of total and fecal coliforms as well as counts of phytoplankton and certain physiological groups of bacteria (proteolytic, amylolytic, lipolytic), we have observed no drastic quantitative or qualitative impacts on the environment other than very local ones. This is, of course, pleasing but cannot be regarded necessarily as a true synopsis of the ecological impact of these additions on the total ecosystem. We should not extrapolate lack of effect on a few to many. Some in situ studies of nitrifying bacteria under thermal enrichment (Pierce and White, 1973; Schwert and White, 1973) have shown an apparent correlation between temperature and nitrification. In another study, a modified BOD test was used to correlate activity of ammonium-oxidizing bacteria with temperature (Roch and Kaffka, 1972). Surely the types of bacteria involved in these and other transformations (Burchard, 1971; Colwell, 1972) are ecologically important yet rarely included in routine monitoring studies; usually, a very small inventory of the total microbial population is considered. Perhaps representatives of the classical producing, consuming, and transforming members of the ecosystem deserve attention if such a cross section could be subject to mutual agreement and if one takes care in assigning labels (Bunt, 1968).

The aquatic environment consists of mixed cultures of a highly competitive nature and biochemical diversity cited above. The addition of a pollutant causes change in the external environment and may lead to stimulation or inhibition of the indigenous populations. The effects may be obvious, in terms of blooms or mass mortalities, or more subtle unless carefully monitored. This could well extend to the study of specific pollutants as chemotactic agents (Adler et al., 1973; Chet et al., 1973) and their influence on biotransformation.

General methods and concepts in organic mineralization continue to be advanced (Hood, 1970; Pomeroy, 1970) and will affect changes in our approach to the microbial transformation of carbon compounds. Of parallel interest are the rates of uptake and release of ecologically significant phosphorous-containing molecules (Johannes, 1964; Satomi and Pomeroy, 1965; Abbott, 1969). Similarly, studies on the rate of transformation of nitrogen compounds (Goering and Dugdale, 1966; Yoshida and Kimata, 1969) are necessary. The ability to detect the various types of nitrogen bacteria involved (i.e., Finstein, 1968; Kawai and Sugahara, 1971) as well as activity under different conditions (Carlucci and McNally, 1969) form a mandatory base for continued work. We are only beginning to detect competition for such common compounds as urea (Remsen et al., 1972) and amino acids (Burnison and Morita, 1973). In addition, the sulfur cycle (Kellogg et al., 1972) represents a complex series of chemical interactions. Only recently have certain groups of participant bacteria been studied closely (Truper et al., 1969; Tuttle and Jannasch, 1972, 1973) and specific econiches continue to be revealed (Fenchel and Riedl, 1970). Some problems in methods for enumeration have appeared (Mara and Williams, 1970).

Given these few examples ot classical element cycling concepts, it must be admitted that our sum knowledge of the intricacies involved is limited. And we know even less of the effects of various pollutants on the activities of the organisms concerned. For basic studies, the enrichment or selective culture technique (Schlegel and Jannasch, 1967) is a powerful tool for getting at specific metabolic types of organisms in the entire biomass. While a very old technique, a wide variety of individual methods are available (Aaronson, 1970; Rodina, 1970). For example, the use of enrichment culture in silica gel medium has been used successfully for the detection of petroleumlytic bacteria in seawater (Seki, 1973). Modification and sophistication to an even greater extent should prove valuable in applying these techniques to particular problems. Rate studies are emphasized in connection with all of these. An occurrence is one thing but the temporal aspect is really what we are after - how fast is something being turned over and to what extent is it being influenced by an environmental additive?

Mention should be made also of the effects of heavy metals on microorganisms. Some examples have been shown for nickel (Cobet et al., 1970), copper (Erickson et al., 1970), lead (Tornabene and Edwards, 1972), and mercury (Nelson et al., 1973). What effect these and other elements have on ecologically important processes is largely unknown but these should be worked into monitoring programs if identified as present as additives. Analytical methods for heavy metal detection are improving; however, the laboratory culture of bacteria in ecologically present quantities of some heavy metals requires very careful manipulation in terms of inorganic contamination of synthetic media.

The EPA Ecological Research Series of publications are valuable sources of methodology for assessing pollution effects on fish and other commercially important species. While much is known of microbial disease in fish (Sinderman, 1966) and shellfish (Sprague, 1971), little information is available on pollution effects on the etiological agents although some data have appeared (e.g., Pippy and Hare, 1969). Relatively "new" diseases of marine life (Haskin et al., 1966; Vendros et al., 1971) and even man (Zeligman, 1972; Jolly and Seabury, 1972) demand revised methodology for detecting the causative organisms and the effects of pollutants on them. Organic and inorganic nutrients and thermal additions would seem to be among the obvious environmental stimulants to be considered in monitoring studies connected with the occurrence of specific pathogens. Here again, refinement of an existing selective culture seems an appropriate place to start.

Often overlooked is the biochemical diversity of microorganisms. In most studies, a marine pseudomonad would not attract special attention yet one such isolate (Buck et al., 1963) is capable of deaminating with subsequent production of ammonia and ichthyotoxicity as well as precipitating calcium carbonate (Greenfield, 1963). These are all ecologically important activities if occurring in situ. Close scrutiny may well show such biological diversity in many bacteria.

Even recognizing quantitative and qualitative variations (Buck, 1974), numbers of microbes (bacteria, phytoplankton) will always be recorded and the debate over their significance will continue. Certainly detection of numbers is more meaningful when some qualitative data accompany them and most useful when some notion of activity or indicator role is clearly assigned. For example, Palmer (1969) suggest that certain genera and species of microalgae can be used to rate water samples for organic pollution levels. Species diversity among intertidal macroalgae shows similar responses (Borowitzka, 1972).

A lesson from the microbiological space program could be useful (Merek and Oyama, 1968); i.e., assume little and apply a broad brush approach at first, then gradually narrowing. A wide spectrum of nutritional types could be sought wherein an environmental additive in question may influence metabolic activity. Also, we may consider things other than bacteria and phytoplankton as being environmentally significant. Yeasts and filamentous fungi offer considerable degradative potential (Ahearn and Meyers, 1973; Cerniglia and Perry, 1973). Yeasts are relatively tolerant of adverse conditions (e.g., salinity) and thus may be present over a wide environmental range (Dzawachiszwili et al., 1964; Fell and van Uden, 1963). Viruses are receiving some attention also in water quality monitoring (Berg, 1972; Shuval and Katzenelson, 1972).

Attached microorganisms represent groups of neglected forms in past studies. Newer data (Bezdek and Carlucci, 1972; Tsyban', 1971) supplement the observations on organic adsorption to bubbles (i.e., Battosingh et al., 1969) and suggest the importance of interfaces as microbial concentrators. The surface microlayers (0.1mm) of seawater may represent an area of high concentration hydrocarbons (Duce et al., 1972) and may have biological effects (Sieburth, 1971). Air-water, solid-water, oil-water, and other interfaces offer intriguing possibilities for microbial concentration.

Carpenter (1972), Wulff and McIntire (1972), and Goering and Parker (1972) have reported on ecologically important activities of attached plant assemblages. We are touching here on a gray area; not gray to ecological thought but often not treated in monitoring. This is the concept of no microorganism being an island but rather a part of the entire microcosm. The impact on biological sequence must be felt in environmental additions. One current interest in eutrophication involves the role of bacteria in producing CO for algal respiration rather than a phosphate and/or nitrate stimulation (Kuentzel, 1969; Lange, 1970). If indeed such a relationship exists, then appropriate monitoring of particular environments, in conjunction with laboratory studies (O'Brien, 1972), will clarify the hypothesis.

We must think too in terms of future use of marine environments for culture of economically valuable animals (or plants) or their products (George Washington University, 1971a, b). The oceans do have size and dilution factors in their favor; however, confinement of small areas for rearing of single species may yield new problems in the form of epizootics and epiphytotics. Continuous monitoring of eggs, larvae, and adults (Johnson et al., 1971) will be required for possible recognition of microbial-induced pathological conditions and other effects (Webber, 1973).

Some years ago, pollution equated with sewage. Today, of course, environmental additives are many and varied. In some cases, technology is wanting for critical identification of certain exotic compounds and some obviously persist for long periods and are widespread (Harvey et al., 1973). We know little of the ultimate fate of these materials or how long their chemical uniqueness will remain a mystery to microorganisms. In fact, even "usual" organic matter is somewhat refractory on the ocean floor (Jannasch et al., 1972; Jannasch and Wirsen, 1973).

Perhaps more interest should be paid to macroorganisms in aquatic habitats. Monitoring of fouling (McNulty, 1970; Ingnatiades and Becacos-Kontos, 1970) and benthic (Reish, 1970; Goodnight, 1973; McDaniel, 1973) organisms have shown some useful applications (and problems) associated with their determination in various situations. The American oyster responds to various levels of PCBs (Lowe et al., 1972) and heavy metals (Calabrese et al., 1973). Biomagnification appears again in the examination of fish bile which may concentrate certain compounds over 1000 fold from water levels (Lech et al., 1973).

Any attempt to detail all available methods for microbiological monitoring would be hopelessly naive. Fortunately, we are updated periodically on technology (e.g., Water Pollution Control Federation, 1973) and reminded occasionally of our task (e.g., Tarzwell, 1962; Wilhm and Dorris, 1968). Overviews of monitoring instrumentation do appear (Ciaccio, 1971; Ballinger, 1972) and, recently, a most valuable volume became available on methodology in microbial ecology (Rosswall, 1973). General (e.g., Mackenthun, 1966; McKee, 1967; Ludwig and Storrs, 1970) and specific (Kneiys, 1971; Norris et al., 1973; Hlavka, 1973) approaches to particualr areas can be consulted also.

Certain uses of photography, particualrly infrared, have great value in water quality monitoring (Merriman, 1970; Funk and Flaherty, 1972). On the organism level, infrared color photography has been employed for detection of unstained microbes in natural habitats (Casida, 1967). Detection and activity of periphytic bacteria can be accomplished with modifications of light microscopy (Bott and Brock, 1970). Development of the scanning electron microscope has revealed an elegant picture of in situ associations (Brooks et al., 1972; Gessner et al., 1972).

Fluorescent techniques are applicable to chlorophyll-containing forms (Wood and Oppenheimer, 1962). Another use involving the fluorescent antibody technique for determination of fecal pollution has provided rapid identification and quantitation of indicator bacteria (Abshire and Guthrie, 1971). Serological tracing has been applied also with microscopy (Glantz, 1968).

Approaches to the study of microbial activity in waters have been reviewed admirably by Fjerdingstad (1971) and Jannasch (1969, 1972). Included are discussions of biomass determinations by ATP measurement and assessments of growth and substrate uptake which include oxygen exchanges and the production of measureable color reactions using TTC. Some interesting in situ applications have appeared on growth rates of bacteria in river water and sediment uptake of substrate (e.g., Hendricks, 1972; Wood and Chua, 1973). Autoradiography (Brock and Brock, 1966) may be added as a method for further exploration (see Rosswall, 1973). Gas chromatography offers another new tool for certain processes and environments (Payne, 1973; Francis et al., 1973).

Bioassay of organic compounds (vitamins, etc.) in natural waters (Belser, 1963) has provided valuable data for productivity studies and ecological interactions. Algae not only require growth stimulants (Provasoli, 1963) but also produce them (Carlucci and Bowes, 1970). A provisional algal assay procedure (PAAP; Weiss and Helms, 1971) has been developed to measure the total algal nutrient potential of natural waters by utilizing the waters as a culture medium yielding a final algal biomass as a nutrient indicator. This test has been modified (Cain and Trainor, 1973) to overcome some inadequacies and now represents a simple, fast, reproducible, and field applicable technique. Some studies have shown the value of marine animals as bioassay agents for hydrocarbons in water (LaRoche et al., 1970).

Given the ingenuity and pool of scientific talent available, the above review of existing methodology will predictably be synthesized into less primitive and more meaningful monitoring. Sorensen and Moss (1973) describe in detail some inherent problems in the implementation of the National Environmental Policy Act. They see the process in three steps of increasing complexity; (a) impact identification, (b) prediction of impacts, and (c) evaluation of a specific impact. In all stages, monitoring of some sort will be included, as well as in post-approval activities. The critical point here is that we must not get tied to untested assessment methods. Constant review and evaluation will engender change in methods and/or approach to include introduction of new techniques and the elimination of others where warranted. In addition, some legal (McManus, 1973) and philosphical (Hedgpeth, 1973) aspects of marine disposal activities are available and should be consulted.

Much of our existing methodology may have originated in the blood and bile era of microbiology but with appropriate modification can continue to serve as a useful base. In the field, good surveys of particular areas subject to certain pollutants are available and should be employed. Techniques of constant sampling and recording, automated analyses, and data storage and retrieval do exist and can be utilized together with streamlined, tailored laboratory procedures. Chemical data are also appropriate and can effectively compliment the use of microorganisms in monitoring the marine ecosystem.

With regard to microorganisms, we may pose a number of questions: (a) does an environmental additive interfere with the inherent capabilities of the microflora (nitrification, proteolysis, lipolysis, etc.), (b) does the resident microflora have the ability to degrade the additive or is the additive totally recalcitrant, (c) if degraded, is the additive completely mineralized to innocous end products, and (d) does the biotransformation produce more toxic or stimulative compounds (ammonia, methylmercury, trimethylarsine)?

The diversity alluded to previously can be assumed only up to a point. Microorganisms could well be the most sensitive indicators of environmental alteration and can be both used and abused. If we design our monitoring techniques properly, we may eventually learn more about the quality of the marine environment and probably have it available sooner.

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MONITORING SYSTEMS AND THEIR USERS: IS THERE A RELATIONSHIP?
(What's It All About, Alfie?)

D. F. Squires *

When I agreed to speak to you this afternoon, I told your seminar chairman that I was far from qualified as an authority on environmental monitoring. Perhaps that is why I am here--late in the session. This speech's title for print has the usual formal flavor. The real title is, "What's It All About, Alfie?"

Let me begin by stating that monitoring our environment in a continued, sustained, coordinated, thoughtful way is probably one of man's most necessary activities. We live in an age of environmental awareness. A new crop of voters is coming of age-those to whom Earth Day was a call to arms to rectify the past sins of their elders. We must not forget that to those young students--in high school or younger--the environmental crisis is very real and has made a strong impression. When, for us older types under intense social pressures, our way of life and our standard of living are threatened by energy problems, it is easy for us to compromise the environment and to forget conveniently about those dramatic issues. To a younger generation, letting up on pollution abatement procedures may seem a cop out--and they may not agree. This situation may lead to a new confrontation, demanding from the scientific community data of a new kind. The scientific community must be prepared to understand--and to interpret--what is happening in the natural world around us.

My current home is on the east coast, in New York State. Over 30 years ago changes began to occur in the coastal waters of Long Island--and in the life sustained by those waters. One of the first indicators of change was an increase of starfish in Long Island Sound. Those predators, coupled with a disastrous sequence of storms, resulted in failure of the oyster industry. Change persisted. Some of the flounder population began to go; other fisheries became marginal or collapsed. Lobsters, it is true, became more abundant; and the eel grass, Zostera, recovered from a ruinous setback by disease, bringing with its regrowth stronger shellfish populations. Change persists. That change is the catalyst of a new, massive, and probably rationally unresolvable conflict between recreational and commercial fishing interests.

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* N.Y. State Sea Grant Program

Now recall the very rough sequence of events I have sketched. Causation for most is not known. Disease in the Zostera is documented; but about the rest, we have to surmise--guess. In the conflict raging bitterly between sports and commercial interests, facts are notably lacking. Some attribute diminishing fish populations to long-term oceanic shifts, others to overfishing. Everybody blames "pollution." All are probably causative factors of the changes in population structure. Fingers are pointed, accusations are leveled, much heat is generated, but very little light is shed.

Alan Longhurst and his colleagues (1) recently called for "deliberately mounted and well-sustained ocean monitoring operations." They make important points:

1. That "pollution monitoring schemes, in the ocean or elsewhere, can only succeed if the natural effects of the changing physical environment are both understood and monitored continuously and indefinitely";
2. That "natural fluctuations in animal populations have already been ascribed incorrectly to the effects of pollutants";
3. That "a serious impact on the environment [could] pass unnoticed through ignorance of natural population instability."

Longhurst and his colleagues document their stance with eloquent examples of biological fluctuations in ocean populations: the fin-fish collapse on the east coast in the 19th century, the complex instabilities of "El Ninos," Pacific sardine fluctuations, and others.

Their point is that when we judge man's impact on the ocean we must avoid the twin pitfalls of:

1. Assuming that the ocean's vastness, its chemical buffering system, and the complexity of the food web will render it capable of absorbing insult after insult;
2. Regarding populations of marine organisms as inherently stable and thinking of changes in them as solely the result of man's intervention.

To cry "Eco-catastrophe!" prematurely is to create another Amchitka. To prophesy the end of the world is surely the way to lose the attention of one's audience. We must beware of crying wolf--or, to cite a recent example, Acanthaster. The "Crown-of Thorns" was a cause celebre, aptly named for its effect on many members of our society.

Up went the rallying cry, "Save our coral reefs from the depradations of the starfish!" But were we witnessing a subtle effect of man's intrusion by insecticides and other pollutants? Or were we, as now seems the case, witnessing a population explosion of a heretofore rare species? We lose credibility with each false clarion call.

Let me turn to another concern which may be new to you. Technocrats no longer stalk the halls of power in Washington. Consumerism has arisen in the land. Science has fallen from the favored position, although its successor has not been chosen. Our society is skeptical of the technological fix, of the knowledgeable but distant technologist with all the answers. We scientists are being pushed to move in new directions. As demands upon science change, so must the system for meeting those demands. William Baker, president of the Bell Telephone Laboratories, testified to the House Committee on Science and Astronautics recently. He cited the change in demand from performance systems to service-oriented economic systems. The hardware of the 1940's, 1950's, and 1960's does not meet the 1970's challenge to science and engineering to solve problems in the service of man--problems of ecology, universal health care, public transport, costly food production and distribution--problems with a huge consumer factor.

You who are engaged in environmental monitoring are serving new masters: you are answerable to a very broad constituency. Are you aware of them? Science for science's sake is no longer enough to pay the way. Are you becoming alert to the needs and the frailties of the new masters? Let me pose some questions and then relate a case study.

Do people understand what you are doing when you monitor the environment--when you model the environment? Do they understand the limitations? Are you telling the taxpayer footing the bill all that he should know? Is it really getting through to him?

I submit that the answer to all is No! To illustrate, I am going to cite from a recent report by one of New York's Sea Grant extension agents on the high water problems on the Lake Ontario shoreline. I should have said, "I'll share this report with you" - That is extensionese for, "You listen while I talk." I will admit at the outset that the monitoring system and model involved are primitive compared to the weighty issues you have been discussing, but the social interactions are real, important, and critical to the future of monitoring programs.

In the winter of 1972-73--this past winter--there was weak ice cover on Lake Ontario with few periods of strong, hard freeze. The usual coastal buffer, a thick ice sheet, was not present. During January and February coastal erosion was greater than usual and breakwaters were undercut. When high winds on March 18 and 19 brought storm waves to the southern shores, extensive erosion, wave overwash and flooding occurred--all exacerbated by the eroded breakwaters. Lake levels in Lake Ontario then were at least one and a half feet lower than the maximal levels reached later, in the summer. By April and May, the winter's high precipitation in the upper Great Lakes caused rapidly rising lake levels. The situation reached serious proportions in the summer. Public conception of these events is now blurry but was confused even in May. The flooding resulting from storm waves merges, in their minds, with flooding from high water levels, despite the fact that recompense depends in large part upon the distinction between the two. Memories are short, hindsight is not always clear, cause and effect often aren't linked. After all, most coastal dwellers assume that when they develop land, erosion will somehow cease.

Now, all these issues have been further clouded by controversy over control of lake levels and the Corps of Engineers' "Operation Foresight." Although Operation Foresight had been announced as a dike system to protect against flooding, it became an erosion control project. Slippage due to technical factors, union problems, and lack of easements from property owners delayed the project from March until September and did not protect against flooding. The Corps of Engineers, relatively blameless in this connection, has been caught by the failure of Operation Foresight. The entire episode is now viewed by many citizens as a federal conspiracy to maintain high lake levels for the benefit of commerce and the electrical generation industry.

This idea is reinforced by the fact that the Lake Levels Control Board is chaired by the Corps. The Board regulates lake levels based on examination of three-month precipitation levels, but the model does not include the storage capacity of the upper lakes. Only 20 percent of Lake Ontario's water is derived from directly received precipitation.

Some people now charge that for the Corps of Engineers to have announced Operation Foresight in December of 1972, the Corps must have perceived the high water threat as early as the summer of 1972. Therefore: (a) high levels reached in the summer of 1973 were quite preventable and (b) were the result of a federal-commercial conspiracy so that (c) disaster relief programs are in fact a payoff. Reason has little sway in such a hotly fired controversy.

The need for "getting through to the audience" is obvious.

1. Coordinated information directed towards local and county governments, perhaps by Sea Grant's advisory

service agents, could have pointed up the increased potential for storm damage because of ice-free conditions.

2. Weather data collection and dissemination could be vastly improved. It is now sent to Cleveland for processing, and comes back to Rochester as forecasts. No distribution is made to Civil Defense or other coordinative agencies.
3. The predictive model for lake levels can be improved by incorporating data on storage in the upper lakes.

Let me summarize. Public opinion and facts are not always identical. We all need to work hard--very hard--at improving public understanding of what we are doing and what our limitations are. I have tried to make several points today. You in monitoring systems, we in other branches of science, need to give a lot of thought to the people we serve, and to their information needs--their education. A new generation, more knowledgeable and much more skeptical than the old, is with us. While a compelling case can be made for monitoring systems of long duration, we must share information we already have which can be used in meeting daily disasters, and at the same time guard against crying wolf. I'm speaking not of doing a public relations job, but of making really thoughtful attempts to educate our ultimate consumer, the public. Our most urgent task is to get thoroughly in touch with the users of environmental monitoring systems--and I do not mean the data banks of this world, another subject, one I will carefully avoid.

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APPENDIX A - AGENDA

SEMINAR ON METHODOLOGY
FOR
MONITORING THE MARINE ENVIRONMENT

Time:	October 16 - 18, 1973
Place:	Washington Plaza Hotel Fifth Avenue at Westlake Seattle, Washington
Chairman:	S. Sid Verner EPA, Office of Research and Development

Tuesday, October 16, 1973

7:45 AM	Registration
8:15 AM	Introduction and Theme Dr. Stanley Greenfield Assistant Administrator for Research and Development
	Session A Basic Framework and Field Applications Moderator: Don Baumgartner, NERC-Corvallis
8:30 AM	Mathematical Modeling as a Framework for Coastal Monitoring R. J. Callaway, NERC-Corvallis
	Turbidity as a Water Quality Parameter R. Austin, Scripps
	Surface Slick Sampling and Analysis -- W. Rittall, NERC-Corvallis
	An Integral Shipboard Surveillance and Analysis Program S. Pavlou, U. of Wash.

10:30 AM	Coffee Break
11:00 AM	Discussion
12:00 PM	Lunch
	Session B Monitoring for Pollutants Moderator: Pat Parker, U. of Texas
1:30 PM	Pesticides
	Methods and Problems in Analysis of Pesticide in the Estuarine Environment A. J. Wilson, Jr., GBERL
	Problems in Monitoring Data Interpretation P. A. Butler, GBERL
3:30 PM	Coffee Break
4:00 PM	Metals
	Methods for Analysis in Sea Water D. H. Kampbell, EPA-Region II
	Metals and Biological Availability in the Marine Environment D. K. Phelps, NMWQL
6:30 PM to 8:00 PM	No Host Mixer

Wednesday, October 17, 1973

	Session B (Continued)
8:00 AM	Petroleum Products
	Recent Work in Monitoring Petroleum Pollution of the Ocean M. H. Feldman, NERC-Corvallis
	Summary of Recent Studies on Biological Effects of Crude Oils and Oil-Dispersant Mixtures to the Red Sea (Israel) Macrofauna R. Eisler, NMWQL

10:00 AM Coffee Break

10:30 AM Thermal Pollution

Problems Using Marine Waters for Industrial Cooling
J. C. Prager, NMWQL

11:30 AM Lunch

1:00 PM Nutrients

Absorption of Orthophosphates on Pyrex, Soft Glass, Polyetheylene and PVC in Water
D. Krawczyk, NERC-Corvallis

Development of a Standard Marine Algal Assay Procedure for Nutrient Assessment
D. T. Specht and W. E. Miller, NERC-Corvallis

3:00 PM Coffee Break

3:30 PM Radionuclides

Monitoring for Radionuclides in the Marine Environment
B. Kahn, NERC-Cincinnati

Methods for Monitoring Radioactivity in Aquatic Biota
V. A. Nelson, W. R. Schell, A. H. Seymour, U. of Wash.

5:30 PM Adjourn

Thursday, October 18, 1973

Session B (Continued)

8:00 AM Sludges and Benthic Material

The Properties and Composition of Sewage Sludges
R. B. Dean, J. E. Smith, Jr., NERC-Cincinnati

Making Bioassays of Marine Ecology Relevant
R. Nadeau, EWQRL

10:00 AM Coffee Break

10:30 AM Dredge Spoils

Methods for Monitoring Dredge Spoils
L. S. Slotta, OSU

An Analysis of the Application of Diversity Indices and Other
Measures to the Problem of Quantifying Environmental Change
R. Swartz and A. J. McErlean, OEGC

12:30 PM Lunch

Session C
Microbiological Aspects
Moderator: Len Guarraia, OAWP

1:30 PM Sampling Methods for Microbiological Analyses
R. R. Colwell U. of MD

Microbiological Methods for Monitoring Marine Waters for
Possible Health Effects
V. J. Cabelli, F. T. Brezenski, A. P. Dufour, M. A. Levin, EPA

Methods for Monitoring the Marine Environment for Ecologically
Important Marine Organisms
J. D. Buck, U. of CT

3:30 PM Coffee Break

Session D
Monitoring Requirements

4:00 PM Monitoring Systems and Their Users: Is There a Relationship?
D. F. Squires, NY State Sea Grant Program

Adjourn

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